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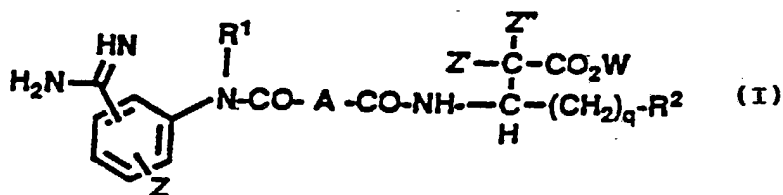
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(54) Title: SUBSTITUTED β -AMINO ACID DERIVATIVES USEFUL AS PLATELET AGGREGATION INHIBITORS



(57) Abstract

Novel substituted beta-amino acid derivatives having general formula (I), are provided, in which e.g. R² is selected from the group consisting of hydrogen, lower alkyl radicals, lower alkenyl radicals, lower alkynyl radicals, alicyclic hydrocarbon radicals, aromatic hydrocarbon radicals, wherein said radicals are optionally substituted with hydroxy, lower alkoxy, lower alkyl, halogen, nitro, cyano, azido, ureido, ureylene, carboxyl or carbonyl derivatives, trifluoromethyl, acyloxy, alkylthio, arylthio, alkylsulfenyl, arylsulfenyl, alkylsulfonyl, arylsulfonyl, amino, alkylamino, trialkylsilyl, aminosulfonyl, dialkylamino, alkanoylamino, aroylamino, phenyl, naphthyl, lower alkynyl which are optionally substituted with one or more of the following: halogen, nitro, lower alkoxy, lower alkyl, trialkylsilyl, azide and phenyl; the invention also pertains to pharmaceutical compositions comprising such derivatives.

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SUBSTITUTED β -AMINO ACID DERIVATIVES
USEFUL AS PLATELET AGGREGATION INHIBITORS

5 This application is a continuation-in-part of
U.S. Application Serial No. 07/866,933 filed April 10,
1992 which in turn is a continuation-in-part of U.S.
application Serial No. 07/777,811 filed October 15,
1991, now abandoned.

Background of the Invention

10

Field of the Invention

This invention pertains to substituted β amino acid
derivatives which inhibit platelet aggregation.

15

Related Art

Fibrinogen is a glycoprotein present as a
normal component of blood plasma. It participates in
platelet aggregation and fibrin formation in the blood
20 clotting mechanism.

Platelets are cellular elements found in whole
blood which also participate in blood coagulation.
Fibrinogen binding to platelets is important to normal
platelet function in the blood coagulation mechanism.
25 When a blood vessel receives an injury, the platelets
binding to fibrinogen will initiate aggregation and form
a thrombus. Interaction of fibrinogen with platelets
occurs through a membrane glycoprotein complex, known as
gpIIb/IIIa; this is an important feature of the
30 platelet function. Inhibitors of this interaction are
useful in modulating or preventing platelet thrombus
formation.

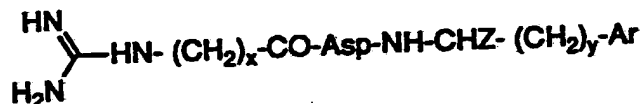
It is also known that another large
glycoprotein named fibronectin, which is a major
35 extracellular matrix protein, interacts with fibrinogen
and fibrin, and with other structural molecules such as
actin, collagen and proteoglycans. Various relatively

large polypeptide fragments in the cell-binding domain of fibronectin have been found to have cell-attachment activity. (See U.S. Patents 4,517,686; 4,589,881; and 4,661,111). Certain relatively short peptide fragments from the same molecule were found to promote cell attachment to a substrate when immobilized on the substrate or to inhibit attachment when in a solubilized or suspended form. (See U.S. Patents 4,578,079 and 4,614,517).

10 In U.S. Patent 4,683,291, inhibition of platelet function is disclosed with synthetic peptides designed to be high affinity antagonists of fibrinogen binding to platelets. U.S. Patent 4,857,508 discloses tetrapeptides having utility as inhibitors of platelet aggregation.

Other synthetic peptides and their use as inhibitors of fibrinogen binding to platelets are disclosed by Koczewiak et al., Biochem. 23, 1767-1774 (1984); Plow et al., Proc. Natl. Acad. Sci. 82, 8057-8061 (1985); Ruggeri et al., Ibid. 83, 5708-5712 (1986); Ginsberg et al., J. Biol. Chem. 260 (7), 3931-3936 (1985); Haverstick et al., Blood 66 (4), 946-952 (1985); and Ruoslahti and Pierschbacher, Science 238, 491-497 (1987). Still other such inhibitory peptides are disclosed in EP Patent Applications 275,748 and 298,820.

U.S. Patent 4,879,313 discloses compounds useful as inhibitors of platelet aggregation having the formula:



35

wherein

x = 6 to 10,

y = 0 to 4,

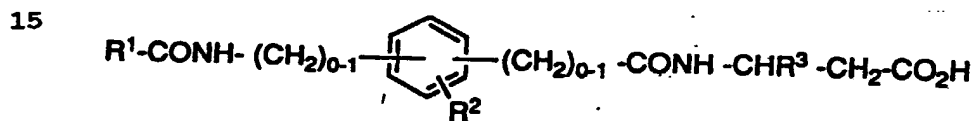
z = H, COOH, CONH₂ or C1-6 alkyl,

5 Ar = phenyl, biphenyl or naphthyl, each substituted with 1 to 3 methoxy groups, or an unsubstituted phenyl, biphenyl, naphthyl, pyridyl or thienyl group,

and

10 Asp = aspartic acid residue.

European Patent Application 372,486 discloses N-acyl β amino acid derivatives of the formula:



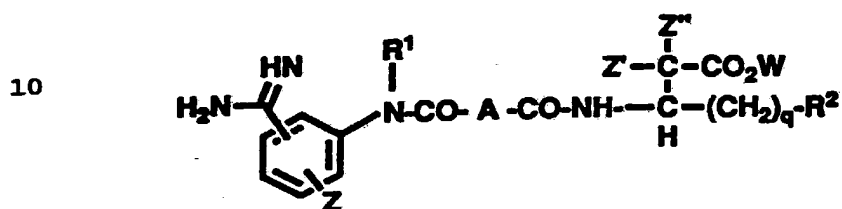
20 and their salts. Said compounds are useful for inhibiting platelet aggregation in the treatment of thrombosis, stroke, myocardial infarction, inflammation and arteriosclerosis, and for inhibiting metastasis.

25 European Patent Application 381,033 discloses amidino or guanidinoaryl substituted alkanolic acid derivatives useful for the treatment of thrombosis, apoplexy, cardiac infarction, inflammation, arteriosclerosis and tumors.

30 European Patent Application 445,796 discloses Acetic Acid derivatives useful as a ligand for adhesive proteins on blood platelet. As such these compounds are useful to modulate and/or inhibit platelet aggregation.

Summary of the Invention

In accordance with the present invention novel substituted β amino acid derivatives are provided which modulate and/or inhibit platelet aggregation. These novel inhibitor compounds can be represented by the following chemical formula.



pharmaceutically acceptable salt, ester or prodrug thereof,

15 wherein R^1 is selected from the group consisting of hydrogen, lower alkyl radicals, lower alkenyl radicals, aromatic hydrocarbon radicals, alicyclic hydrocarbon radicals, benzyl radicals, phenethyl radicals, wherein said radicals are optionally substituted with halogen, lower alkoxy, hydroxy and lower alkyl;

R^2 is selected from the group consisting of hydrogen, lower alkyl radicals, lower alkenyl radicals, lower alkynyl radicals, alicyclic hydrocarbon radicals, aromatic hydrocarbon radicals, wherein said radicals are optionally substituted with hydroxy, lower alkoxy, lower alkyl, halogen, nitro, cyano, azido, ureide, ureylene, carboxyl or carbonyl derivatives, trifluoromethyl, acyloxy, alkylthio, arylthio, alkylsulfenyl, arylsulfenyl, alkylsulfonyl, arylsulfonyl, amino, alkylamino, trialkylsilyl, aminosulfonyl, dialkylamino, alkanoylamino, aroylamino, phenyl, naphthyl, lower alkynyl which are optionally substituted with one or more of the following: halogen, nitro, lower alkoxy, lower alkyl, trialkyl silyl, azide and phenyl.

A is selected from the group consisting of lower alkyl radicals, lower alkenyl radicals, lower alkynyl radicals, and alicyclic radicals, wherein said radicals are optionally substituted with hydroxyl, lower alkoxy, lower alkyl, halogen, alkoxycarbonylalkyl, amino, alkylamino, dialkylamino, acylamino, alkylthio, sulfonyl, and aromatic hydrocarbons which are optionally substituted with halogen, nitro, lower alkoxy and lower alkyl;

W is selected from the group consisting of hydrogen, lower alkyl radicals, lower alkenyl radicals, lower alkynyl radicals, alicyclic hydrocarbon radicals and aromatic hydrocarbon radicals, wherein said radicals are optionally substituted with hydroxyl, lower alkoxy, lower alkyl, halogen, nitro, amino, acyloxy, and phenyl and naphthyl which may be optionally substituted with halogen, nitro, lower alkoxy, and lower alkyl;

Z, Z', Z'' are independently selected from the group consisting of hydrogen, lower alkyl radicals, halogen, alkoxy, cyano, sulfonyl, carboxyl, alkoxycarbonyl, and hydroxyl radicals;

q is an integer from 0 to about 6; and with the proviso that when A is trimethylene and q is 0 then R₂ is not hydrogen, methyl radical or phenyl radical and also that when A is trimethylene and q is 1 then R₂ is not hydrogen.

The above is preferably the compound, pharmaceutically acceptable salt or ester thereof.

R¹ is preferably hydrogen, alkyl and benzyl radicals, R¹ is more preferably hydrogen.

R² is preferably lower alkenyl and lower alkynyl. R² is more preferably vinyl, ethynyl, alkylphenylsulfonyl or alkylmethoxycarbonyl.

A is preferably lower alkyl which may be substituted as defined above. A is more preferably methylene, ethylene, propylene, cyclopropylene; most

preferably A is ethylene or cyclopropylene; even most preferably A is ethylene.

W is preferably hydrogen or lower alkyl radicals; more preferably W is hydrogen or ethyl.

5 Z, Z', Z" are preferably independently selected from the group consisting of hydrogen, chloro, benzyl, methyl, ethyl, hydroxyl, methoxy; more preferably, Z, Z', Z" are selected from hydrogen, benzyl, ethyl; most preferable, Z, Z', Z" are hydrogen.

10 q is preferably an integer of 0 to about 4; more preferably 0 to about 2; most preferably 0 or 1. Most preferred 0.

 It is another object of the invention to provide a novel pharmaceutical composition comprising
15 compounds of the formula I useful in inhibiting or modulating platelet aggregation or the like, particularly in inhibiting or modulating platelet aggregation by administering an amount between .5 mg/kg to 10 mg/kg preferably 3 mg/kg to an animal in need
20 thereof.

 It is still another object of the invention to provide a method to therapeutically inhibit or modulate platelet aggregation or the like in a mammal in need of such treatment comprising a compound of the formula I in
25 unit dosage form.

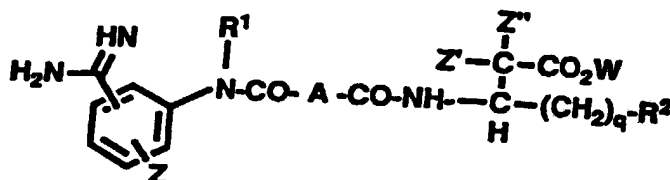
 Many other objects and purposes of the invention will be clear from the following detailed description of the invention.

Detailed Description of the Invention

A preferred embodiment of the present invention is a compound of the formula I:

5

10



pharmaceutically acceptable salt, ester or prodrug thereof,

15

wherein R_1 is selected from hydrogen, lower alkyl radicals of 1 to about 6 carbon atoms, lower alkenyl radicals of 1 to about 6 carbon atoms, aromatic hydrocarbon radicals, alicyclic hydrocarbon radicals of 3 to about 6 carbon atoms, benzyl radicals, phenethyl radicals, wherein said radicals are optionally substituted with halogen, lower alkoxy, hydroxy and lower alkyl;

R_2 is selected from hydrogen, lower alkyl radicals of 1 to about 6 carbon atoms, lower alkenyl radicals of 2 to about 6 carbon atoms, lower alkynyl radicals of 2 to about 8 carbon atoms, alicyclic hydrocarbon radicals of 3 to 6 carbon atoms, aromatic hydrocarbon radicals, wherein said radicals are optionally substituted with hydroxyl, lower alkoxy, lower alkyl, halogen, nitro, cyano, azido, ureide, ureylene, amino, trialkylsilyl, alkylsulfonyl, phenylsulfonyl, trifluoromethyl, acetoxy, acetylamino, benzoylamino, carbonyl, carboxyl derivatives, alkylsulfonyl amino, and phenylsulfonyl amino;

35

A is selected from lower alkyl radicals of 1 to about 6 carbon atoms, lower alkenyl radicals of 2 to about 6 carbon atoms, lower alkynyl radicals of 2 to

about 4 carbon atoms, and alicyclic hydrocarbon radicals of 3 to about 5 carbon atoms, wherein said radicals are optionally substituted with hydroxyl, lower alkoxy, halogen, alkylthio and amino;

5 W is selected from hydrogen, lower alkyl radicals of 1 to about 6 carbon atoms, lower alkenyl radicals of 2 to about 6 carbon atoms, alicyclic hydrocarbon radicals of 3 to about 6 carbon atoms, and aromatic hydrocarbon radicals of 6 to about 12 carbon
10 atoms, wherein all of said radicals are optionally substituted with hydroxyl, lower alkoxy, lower alkyl, halogen, nitro, amino, and acyloxy;

 Z, Z', Z" are independently selected from the group consisting of hydrogen, halogen, alkoxy, cyano,
15 sulfonyl, carboxyl, alkoxy carbonyl alkyl, alkoxy carbonyl and lower alkyl radicals; and

 q is an integer from 0 to about 6. R¹ is preferable hydrogen:

 R² is preferable hydrogen lower alkyl radicals
20 of 1 to about 6 carbon atoms and is optionally substituted as defined above. R² is more preferably hydrogen and lower alkyl.

 A is preferably lower alkyl of 1 to about 6 carbon atoms which is optionally substituted as defined
25 above. A is more preferably lower alkyl of 1 to above 6 carbon atoms.

 Z is preferably hydrogen, halogen and lower alkyl, more preferably Z' is hydrogen.

 Z' and Z" are preferably independently
30 selected from hydrogen, lower alkyl and hydroxy.

 Another preferred embodiment of the present invention is a compound of the formula I or a pharmaceutically acceptable salt or prodrug thereof,

 wherein R_i is selected from hydrogen, lower
35 alkyl radicals, lower alkenyl radicals, aromatic hydrocarbon radicals, alicyclic hydrocarbon radicals, benzyl radicals, phenethyl radicals, wherein all of said

radicals are optionally substituted with halogen, lower alkoxy, hydroxy and lower alkyl;

R^2 is selected from hydrogen, lower alkyl radicals, lower cycloalkyl radicals, lower alkenyl radicals, lower alkynyl radicals, phenol radicals, phenyl radicals, naphthyl radicals wherein each radical may have one or more substituents selected from the group consisting of halogen, lower alkyl, lower alkoxy, carboxyl derivatives, nitro, cyano, azido, ureido, ureylene, alkylcarbonyloxy, hydroxyl, alkylamino, alkoxy carbonyl, trialkylsilyl, alkoxyimino, alkylsulfonyl, phenylsulfonyl, alkylsulfonyl amino, phenylsulfonyl amino and amino;

A is selected from lower alkyl radicals, lower cycloalkyl, and lower alkenyl radicals;

W is selected from the group consisting of hydrogen and lower alkyl radicals;

Z, Z', Z" are independently selected from the group consisting of halogen, hydrogen, and lower alkoxy, and alkoxy carbonyl, and alkoxy carbonylmethyl, and lower alkyl radicals;

q is an integer from 0 to about 6. R^1 is preferably hydrogen, lower alkyl, benzyl and phenyl. More preferably R^1 is hydrogen.

R^2 is preferably lower alkyl and may be optionally substituted as defined above.

A is preferably lower alkyl and lower cycloalkyl. A is more preferably lower cycloalkyl.

Z is preferably hydrogen, halogen and lower alkyl.

Z' and Z" are preferably independently selected from hydrogen, lower alkyl and hydroxy.

Q is preferably 0 to about 4, more preferably 0 to about 2, most preferably 1.

It is contemplated that the following compounds should be exemplifying examples:

As utilized herein, the term "lower alkyl", alone or in combination, means an acyclic alkyl radical containing from 1 to about 10, preferably from 1 to about 8 carbon atoms and more preferably 1 to about 6 carbon atoms. Examples of such radicals include methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, pentyl, iso-amyl, hexyl, octyl and the like.

The term "lower alkenyl" refers to an unsaturated acyclic hydrocarbon radical in so much as it contains at least one double bond. Such radicals containing from about 2 to about 10 carbon atoms, preferably from about 2 to about 8 carbon atoms and more preferably 2 to about 6 carbon atoms. Examples of suitable alkenyl radicals include propylenyl, buten-1-yl, isobutenyl, pentenylen-1-yl, 2-2-methylbuten-1-yl, 3-methylbuten-1-yl, hexen-1-yl, hepten-1-yl, and octen-1-yl, and the like.

The term "lower alkynyl" refers to an unsaturated acyclic hydrocarbon radicals in so much as it contains one or more triple bonds, such radicals containing about 2 to about 10 carbon atoms, preferably having from about 2 to about 8 carbon atoms and more preferably having 2 to about 6 carbon atoms. Examples of suitable alkynyl radicals include ethynyl, propynyl, butyn-1-yl, butyn-2-yl, pentyn-1-yl, pentyn-2-yl, 3-methylbutyn-1-yl, hexyn-1-yl, hexyn-2-yl, hexyn-3-yl, 3,3-dimethylbutyn-1-yl radicals and the like.

The term "alicyclic hydrocarbon" or "cycloalkyl" means a aliphatic radical in a ring with 3 to about 10 carbon atoms, and preferably from 3 to about 6 carbon atoms. Examples of suitable alicyclic radicals include cyclopropyl, cyclopropylenyl, cyclobutyl, cyclopentyl, cyclohexyl, 2-cyclohexen-1-ylenyl, cyclohexenyl and the like.

The term "aromatic hydrocarbon radical" means 4 to about 16 carbon atoms, preferably 6 to about 12

carbon atoms, more preferably 6 to about 10 carbon atoms. Examples of suitable aromatic hydrocarbon radicals include phenyl, naphthyl, and the like.

The term "acyloxy" encompasses acyl from 1 to about 8 carbon atoms. Suitable examples include alkanoyloxy, benzoyloxy and the like.

The term "lower alkoxy", alone or in combination, means an alkyl ether radical wherein the term alkyl is as defined above and most preferably containing 1 to about 4 carbon atoms. Examples of suitable alkyl ether radicals include methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy, iso-butoxy, sec-butoxy, tert-butoxy and the like.

The term "carboxyl derivatives" refers to a carboxylic acid derivative, such as the following:

1. ester (COOR)

Wherein R can be lower alkyl, alicyclic hydrocarbon radical or aromatic hydrocarbon radical,

2. amide (CONR'R'')

Wherein R' and R'' are independently selected from the group consisting of hydrogen, linear or alicyclic hydrocarbon, aromatic hydrocarbon radical, hydroxy or alkoxy radical,

25

The term "carbonyl derivative" is defined as:



R is as defined above. X can be oxygen, sulfur or N-R' wherein R' is defined as above. The term halogen means fluorine, chlorine, bromine or iodine.

The term "pharmaceutically acceptable salt" refers to a salt prepared by contacting a compound of formula (I), with an acid whose anion is generally considered suitable for human consumption. Examples of pharmacologically acceptable salts include the

hydrochloride, hydrobromide, hydroiodide, sulfate, phosphate, acetate, propionate, lactate, maleate, oxalate, malate, succinate, and tartrate and citrate salts. All of these salts may be prepared by
5 conventional means by reacting, for example, the appropriate acid with the corresponding compound of Formula I.

Suitable pharmaceutically-acceptable base addition salts of compounds of Formula I, II and III.,
10 include metallic salts made from aluminum, calcium, lithium, magnesium, potassium, sodium and zinc or organic salts made from N,N'-dibenzylethylenediamine, chlorprocaine, choline, diethanolamine, ethylenediamine, meglumine (N-methylglucamine) and
15 procaine.

The term "prodrug" refers to a compound that is made more active *in vivo*.

Total daily dose administered to a host in single or divided doses may be in amounts, for example,
20 from 0.001 to 100 mg/kg body weight daily and more usually 0.01 to 10 mg/kg. Dosage unit compositions may contain such amounts of submultiples thereof to make up the daily dose.

The amount of active ingredient that may be combined with the carrier materials to produce a single
25 dosage form will vary depending upon the host treated and the particular mode of administration.

It will be understood, however, that the specific dose level for any particular patient will
30 depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diets, time of administration, route of administration, rate of excretion, drug combination, and the severity of the particular disease
35 undergoing therapy.

The compounds of the present invention may be administered orally, parenterally, by inhalation spray,

rectally, or topically in dosage unit formulations containing conventional nontoxic pharmaceutically acceptable carriers, adjuvants, and vehicles as desired.

Injectable preparations, for example, sterile
5 injectable aqueous or oleaginous suspensions may be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a nontoxic
10 parenterally acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are
15 conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectable.

20 Suppositories for rectal administration of the drug can be prepared by mixing the drug with a suitable nonirritating excipient such as cocoa butter and polyethylene glycols which are solid at ordinary temperature but liquid at the rectal temperature and
25 will therefore melt in the rectum and release the drug.

Solid dosage forms for oral administration may include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active compound may be admixed with at least one inert diluent such as sucrose
30 lactose or starch. Such dosage forms may also comprise, as in normal practice, additional substances other than inert diluents, e.g., lubricating agents such as magnesium stearate. In the case of capsules, tablets, and pills, the dosage forms may also comprise buffering
35 agents. Tablets and pills can additionally be prepared with enteric coatings.

Liquid dosage forms for oral administration may include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, and elixirs containing inert diluents commonly used in the art, such as water. 5 Such compositions may also comprise adjuvants, such as wetting agents, emulsifying and suspending agents, and sweetening, flavoring, and perfuming agents.

While the compounds of the invention can be administered as the sole active pharmaceutical agent, 10 they can also be used in combination with one or more active pharmaceutical agents. When administered as a combination, the therapeutic agents can be formulated as separate compositions which are given at the same time or different times, or the therapeutic agents can be 15 given as a single composition.

In the structures and formulas herein, the bond drawn across a bond of an aromatic ring can be to any available atom on the aromatic ring.

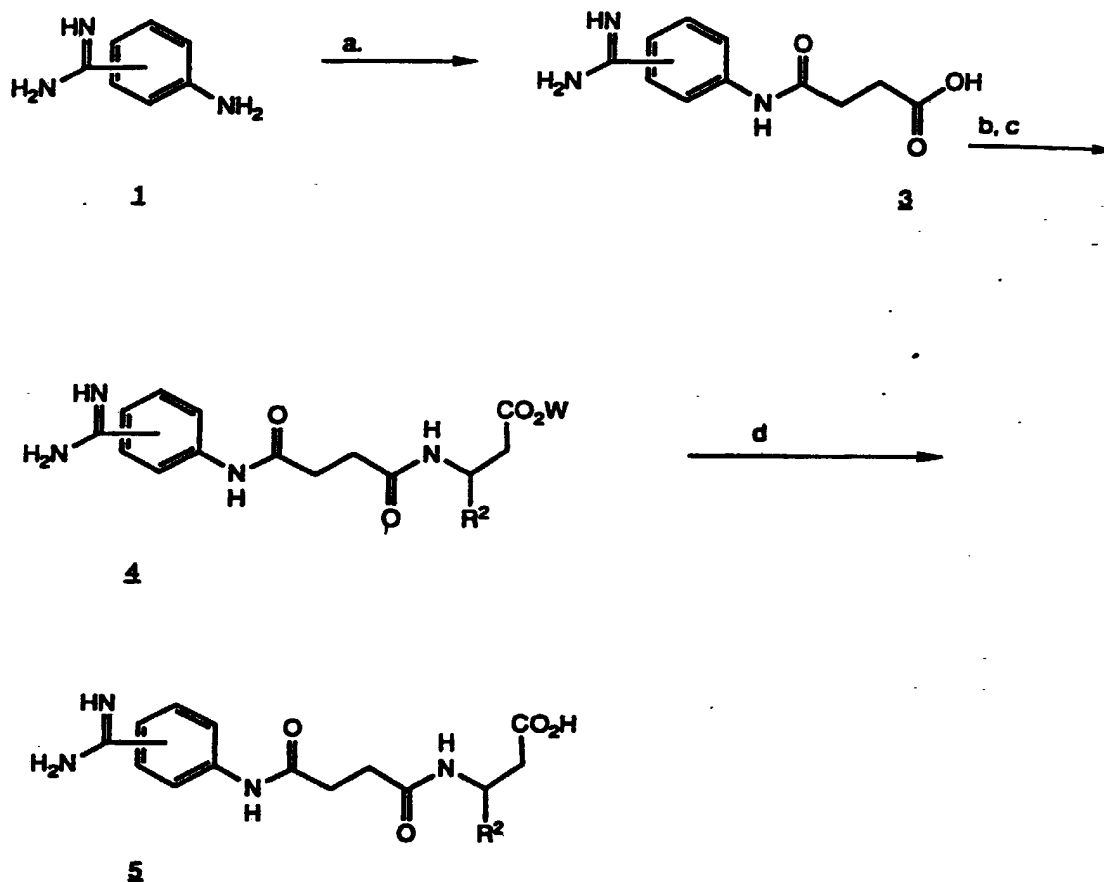
The compounds in this invention can exist in 20 various isomeric forms and all such isomeric forms are meant to be included. Tautomeric forms are also included in the invention. Pharmaceutically acceptable salts of such isomers and tautomers are meant to be included as well.

25 It is also contemplated that Beta amino acids ($H_2N-CHR-CH_2-CO_2H$) used in this invention may be replaced by Homo Beta amino acids ($H_2N-CH_2-CHR-CO_2H$).

The compounds listed above may be prepared by standard synthetic methods combined with methods 30 analogous to solution phase peptide synthesis [see: The Peptides: Analysis, Synthesis, Biology (E. Gross and J. Meienhofer, eds.), Vol. 1-5, Academic Press, New York)], the disclosure of which is hereby incorporated by reference.

35 Five general synthetic sequences are outlined in Schemes 1-5.

SCHEME I

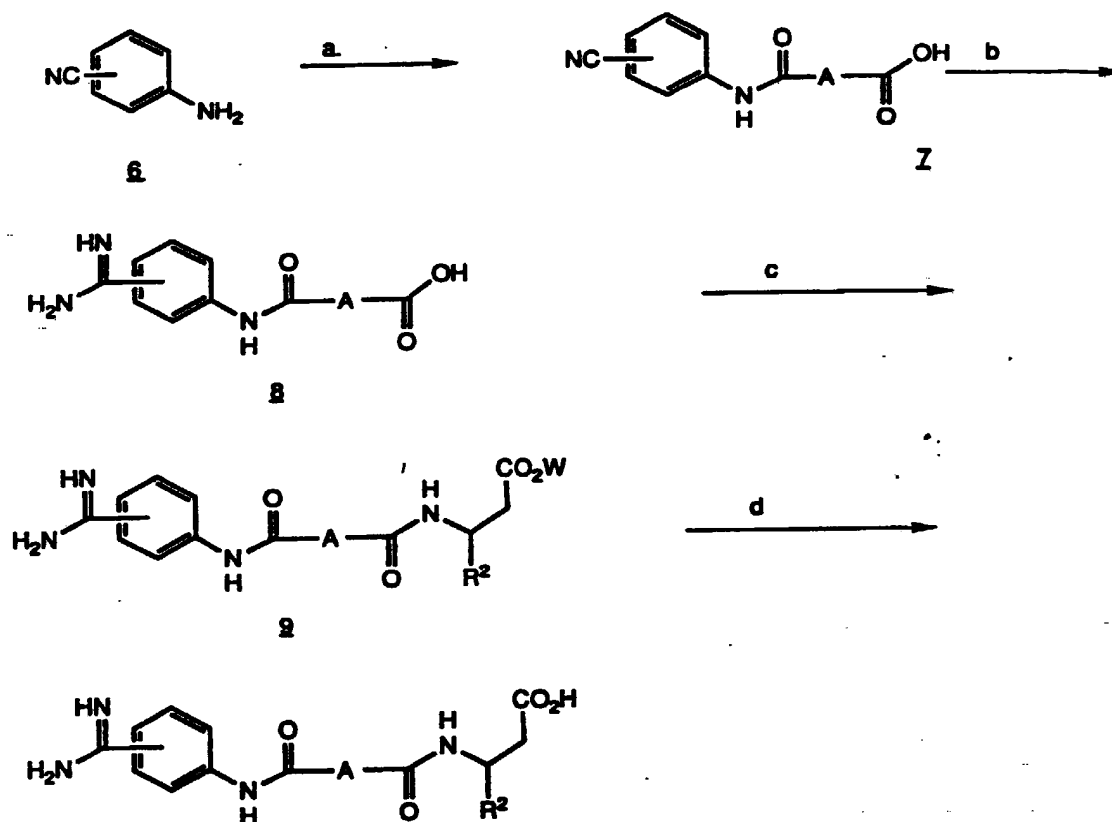


a. Succinic anhydride (2), pyridine, DMAP. b. L-ButOCOCi, NMM. c. β -Alanine derivative
d. NaOH or LiOH.

Wherein W and R² have the values described in formula I

In Scheme I. The aminobenzamidine 1 (i.e., Z is hydrogen) is coupled to an alkanoic, alkenoic (both substituted or not) or alkynoic diacid. An activated form of the diacid is preferentially used. These
5 activated forms include anhydrides, internal anhydride, acid chloride or one of the various activated forms as described in Principles of Peptide Synthesis, Bodansky, 1984, Springer-Verlag, the disclosure of which is hereby incorporated by reference. A highly preferred procedure
10 involves condensation of an anhydride (e.g., succinic anhydride 2) with a salt of aminobenzamidine 1. The reaction is best conducted in a polar solvent such as methylene chloride, acetonitrile, dioxane, dimethylformamide, dimethylsulfoxide or a mixture of
15 such solvents in the presence of an acid binding agent such as sodium, potassium or cesium carbonate, triethylamine, pyridine, sodium hydride, dimethylaminopyridine, diazabicycloundecene, or a mixture of such agents, at temperatures ranging between
20 0°C and 120°C. The final compounds are obtained by coupling of the amidine derivative 3 with a properly protected β -aminoacid. The amide bonds are formed using standard coupling reagents, e.g., dicyclohexylcarbodiimide (DCC), carbonyldiimidazole
25 (CDI), disuccinimidyl carbonate (DSC), benzotriazol-1-yl-oxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP) or isobutyl chloroformate (mixed anhydride method). When the β -amino acid used in the coupling was protected as an ester of the carboxylic
30 acid function (4, W = alkyl, aryl,...), the free acids 5 are obtained by a suitable deprotection method as described by T. H. Greene in "Protective Group in Organic Synthesis", Wiley-Interscience, 1980, the disclosure of which is hereby incorporated by reference.

SCHEME II



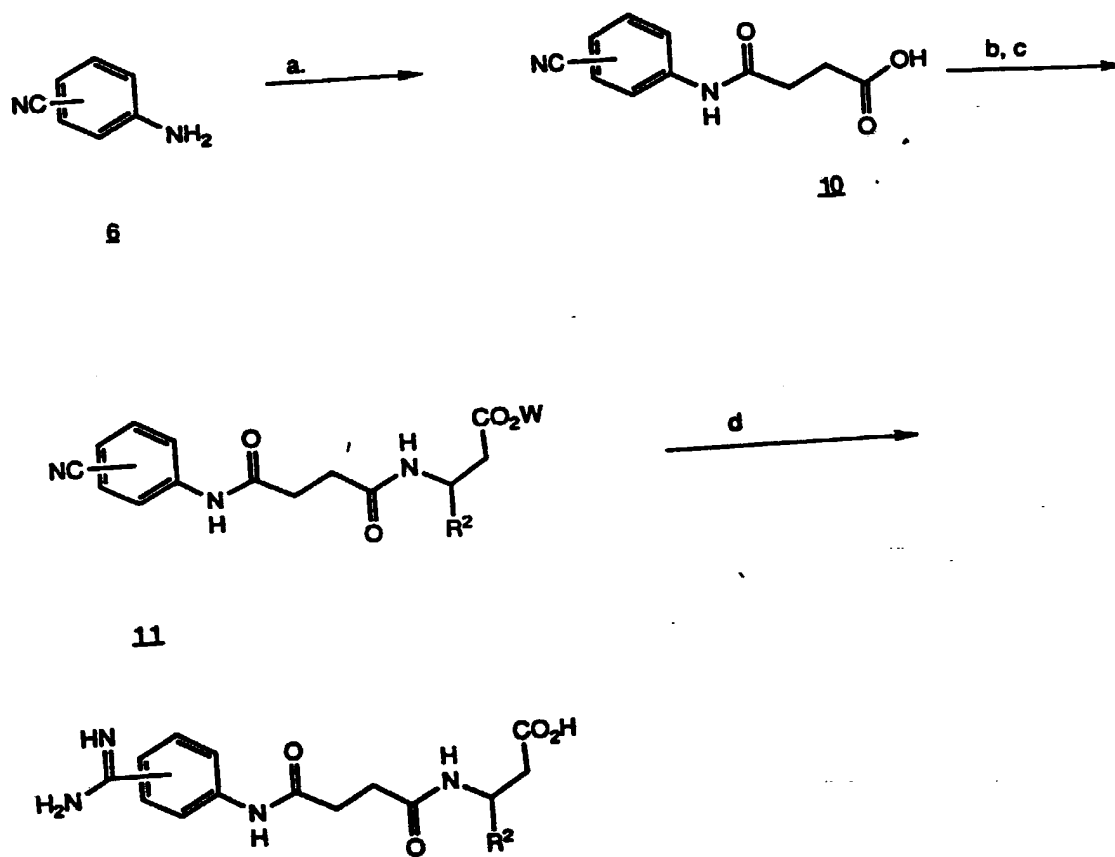
a. Activated diacid. b. H_2S , pyridine; MeI, acetone; NH hexamethyl disilazane in diethyl ether. c. Anhydride
d. Base or acid.

A, W and R^2 have the values described in the general formula.

Alternatively, an aminobenzonitrile 6, can be used for condensation with the desired diacid or diacid derivative. In that case, the nitrile can be converted to the amidine directly or at a later stage. When the aminobenzonitrile is used in the condensation reaction (Scheme II), the cyano group of the resulting intermediate 7 is converted to the amidine 8 via the thioimide in nearly quantitative yield. The thioimide is formed by first treating the cyano compound with hydrogen sulfide (H_2S) followed by alkylation with methyl iodide. Next, treatment of the thioimide with ammonium acetate affords the amidine as the salt (HI). Alternatively, the nitrile 7 can be converted to the amidine 8 by the use of lithium bis(trimethylsilyl)amide in an inert solvent such as diethyl ether [R. T. Boere et al, J. Organomet. Chem., 331, 161-67, (1987)], the disclosure of which is hereby incorporated by reference. The desired compounds are obtained by coupling of the amidine derivative 8 with a properly functionalized β -aminoacid. The amide bonds are formed using standard coupling reagents as described above for Scheme I.

SCHEME III

SCHEME III



a. Succinic anhydride, pyridine, DMAP. **b.** Anhydride mixte, NMM. **c.** α -Allanine derivatives
d. H₂S, pyridine; MeI, acetone; NH₄OAc or Hexamethyl diisilazane in diethyl ether.

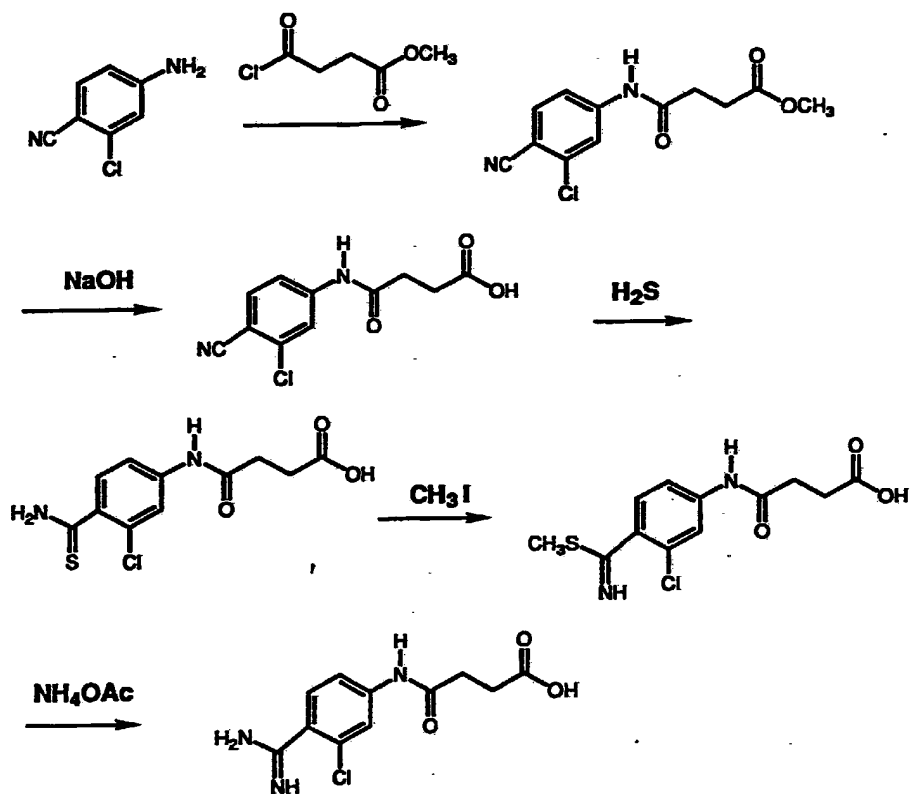
Scheme III illustrates the obtention of derivatives using the amino nitriles as reagents. The cyano group is kept unchanged as a precursor for the amidine function throughout two amide bond forming steps. The first intermediate 10 is directly engaged in a reaction with the desired amino acid. The intermediate 10 is then converted to the benzamidine. A method of choice to produce the amidine function is via the thioimide procedure as described in Scheme II. It is desirable, in Scheme III, to prepare the intermediate 11 as an ester. The most desirable ester is the t-butyl ester which can be deprotected to the acid by contact with a strong acidic medium as HBr/AcOH or trifluoroacetic acid/dichloromethane.

SCHEME IV and V

Substituted aminonitrile can be used to prepare substituted N-aminobenzamidine succinyl derivatives as specifically illustrated in Scheme IV for the chloro derivative 14. The beta amino acids can be either purchased or prepared from commercially available starting materials using known methods as illustrated in Scheme V. The racemic beta aryl beta amino acids can be prepared from the appropriate arylaldehyde, malonic acid, and ammonium acetate as shown in Scheme V - method 1 (Johnson and Livak J. Am. Chem. Soc. 299 (1936)]. The racemic beta alkyl beta amino acids can be prepared from the corresponding alkene and chlorosulfonyl isocyanate (CSI) which goes through the beta lactam intermediate as shown in Scheme V - method 2 [W. A. Szabo Aldrichimica Acta 23 (1977); R. Graf Angew. Chem. Internat. Edit. 172 (1968)]. The beta lactam can be opened to the ethyl ester by treatment with anhydrous hydrochloric acid in ethanol as shown Scheme V. For example, 1,3-butadiene and 3-phenyl-1-propene reacted with CSI to form the beta lactam and following subsequent opening with anhydrous HCl in ethanol were used in Examples 28 and 34 respectively. An alternative method to form racemic beta amino esters is shown in Scheme V method 3.

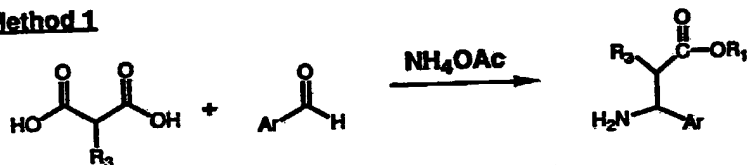
Nucleophiles can be added to 4-benzoyloxy-2-azetidinone to afford a variety of 3-substituted beta amino esters after treatment with anhydrous HCl in ethanol. For example, 1-lithio-2-trimethylsilylethyne was added to 4-benzoyloxy-2-azetidinone to afford the beta amino ester of Example 36 after ring opening [for a similar reaction see: D.H. Hua and A. Verma Tetrahedron Lett. 547-550 (1985) or T. Kametani, Heterocycles Vol. 17 463 (1982)]. In another example, Example 30, 4-benzoyloxy-2-azetidinone was reacted with allyltrimethylsilane under lewis acid catalysis [titanium tetrachloride-K. Prasad et al., Vol. 19 Heterocycles 2099 (1982)]. In Example 28, the cyclopropyl derivative was prepared from the

Scheme IV

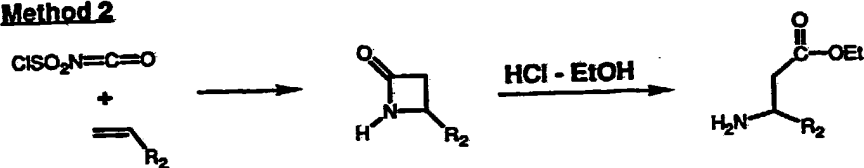


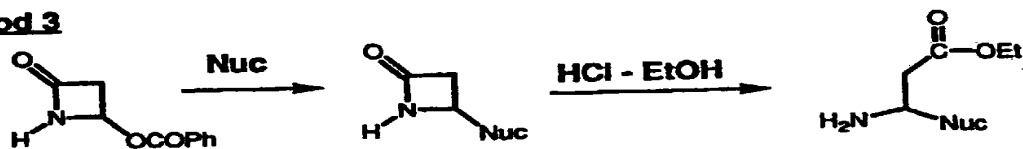
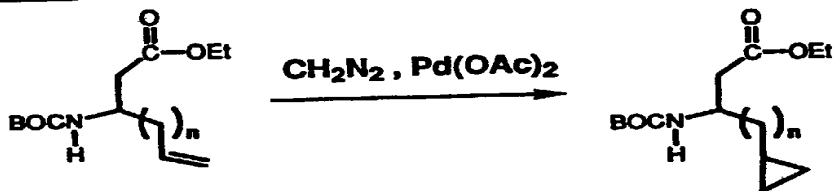
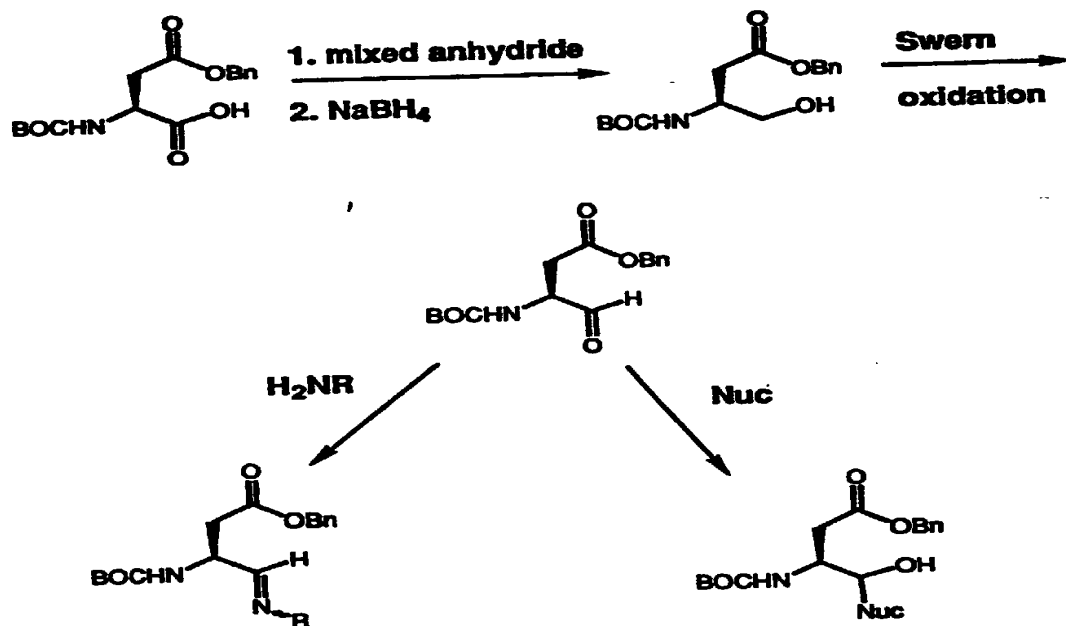
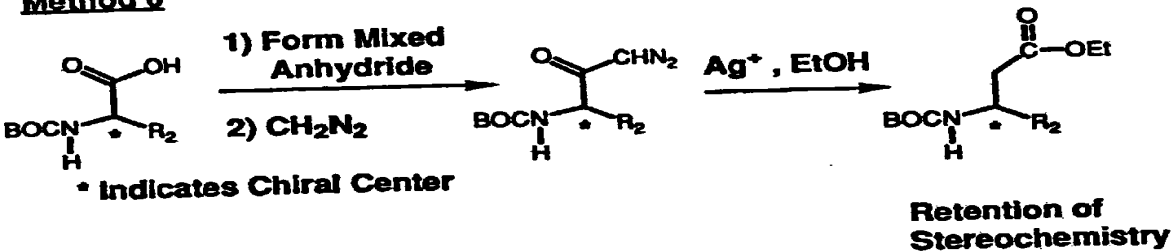
Scheme V

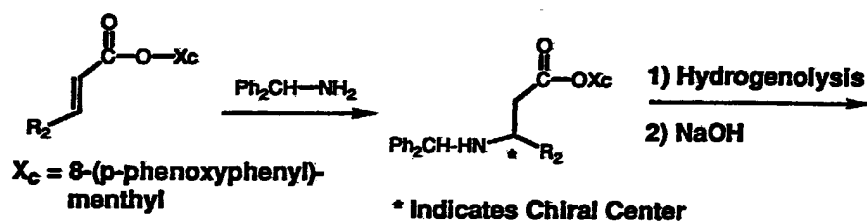
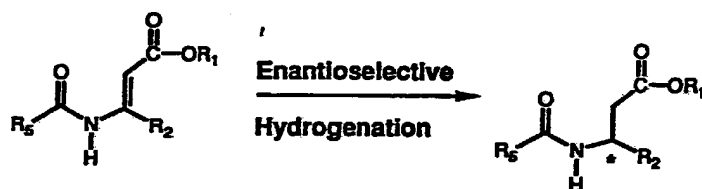
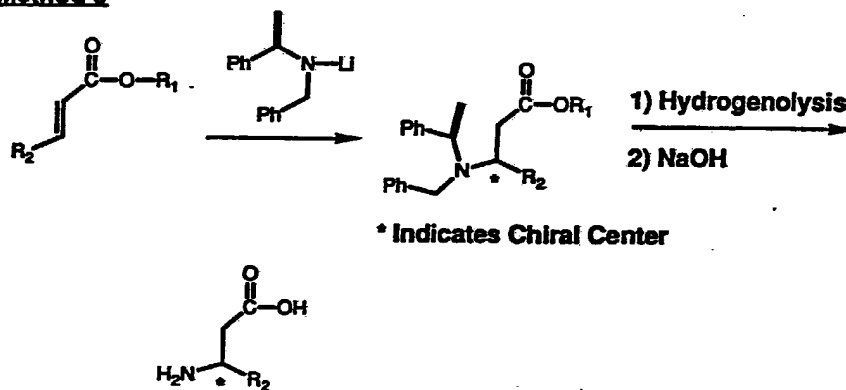
Method 1



Method 2



Method 3**Method 4****Method 5****Method 6**

Scheme V (Cont'd)**Method 7****Method 8****Method 9**

corresponding vinyl compound by treatment with diazomethane and palladium acetate [U. Mande et al., Tetrahedron Lett. 629 (1975)] as shown in Scheme V method 4. The racemic beta amino acids can be resolved using classical methods as described in the literature [E. Fischer, H. Scheibler, R. Groh Ber. 2020 (1910); E. Fischer, H. Scheibler Annalen 337 (1911)].

Chiral beta amino acids can be prepared using many different approaches including the following methods: homologation of the alpha amino acids using an Arndt-Eistert reaction as shown in Scheme V method 5 [Meier and Zeller Angew. Chem. Int. Ed. Eng. 32-43 (1975)] as shown in Scheme F method 3 [M. Rodriguez et al Tetrahedron Lett. 5153 (1990); W. J. Greenlee J. Med. Chem. 434 (1985) and references therein]; from enantiomerically pure precursors obtained from L-aspartic acid [i.e., Scheme V method 6, see: M. Rodriguez Tetrahedron Lett. 923 (1991)]; through the addition of chiral amines to alpha, beta unsaturated esters bearing a chiral auxiliary as shown in Scheme V method 7 [J. d'Angelo and J. Maddaluno J. Am. Chem. Soc. 8112-14 (1986)]; through an enantioselective hydrogenation of a dehydroamino acid as shown in Scheme V method 8 [see: Asymmetric Synthesis, Vol. 5, (J.D. Morrison, ed.) Academic Press, New York, 1985]; through the addition of enantiomerically pure amines to alpha, beta unsaturated esters as shown in Scheme V method 9 [see: S.G. Davies and O. Ichihara Tetrahedron:Asymmetry 183-186 (1991)].

Method 6 of Scheme V was used to obtain a versatile enantiomerically pure aldehyde intermediate. The aldehyde was reacted with methoxylamine to form the oxime which was used in Example 40. The appropriate organometallic was added to the aldehyde to afford the corresponding alcohol.

- The Z substituents, (where Z is hydrogen or halogen, or an alkyl radical or alkoxy radical) can be introduced at the aminobenzonitrile stage. The phenyl group can be halogenated using bromine, iodine, or chlorine. The alkyl group can be introduced by low temperature lithium halogen exchange followed by quenching with the appropriate aldehyde [see: W. E. Parham, C. K. Bradsher Acct. Chem. Res. 300 (1982)], the disclosure of which is hereby incorporated by reference.
- The resulting alcohol can be converted to alkyl by hydrogenolysis [Reductions in Organic Chemistry (M. Hudicky, ed.), John Wiley & Sons, New York, 1984]., the disclosure of which is hereby incorporated by reference. Where Z is hydroxy or alkoxy, such substituents can be introduced by low temperature lithium halogen exchange followed by quenching with electrophilic bis(trimethylsilyl) peroxide $[(\text{TMSO})_2]$ M. Taddei and A. Ricci Synthesis 633-635 (1986)], the disclosure of which is hereby incorporated by reference, which affords the silyl ether. The silyl ether can be converted to the hydroxy derivative by treatment with hydrochloric acid [M. Taddei and A. Ricci ibid]. The hydroxy in the presence of a weak base (K_2CO_3) and an appropriate alkyl halide [$\text{R}_8\text{-Hal}$, Allen C.F. and Gates J.W., Org. Synth. Coll. Vol 2 3 140 (1955)], the disclosure of which is hereby incorporated by reference.] which will form the ester as well. The ester can be selectively cleaved in the presence of the ether with one equivalent of sodium hydroxide.
- For derivatives wherein R^1 is different from hydrogen, such derivatives can be obtained by using an appropriately substituted aminobenzonitrile. For example, the N-methylaminoonitrile can be reacted with 3-carbonmethoxypropionyl chloride to form the required intermediate.

Purification of final compounds is by reverse phase high pressure liquid chromatography (High

Performance Liquid Chromatography Protein and Peptide Chemistry, F. Lottspeich, A. Henschel, K.P. Hupe, eds. Walter DeGruyter, New York, 1981, the disclosure of which is hereby incorporated by reference) or

5 crystallization.

Contemplated equivalents of the general formulas set forth above for the platelet aggregation inhibitors and derivatives as well as the intermediates are compounds otherwise corresponding thereto and having
10 the same general properties wherein one or more of the various R groups are simple variations of the substituents as defined therein, e.g., wherein R is a higher alkyl group than that indicated. In addition, where a substituent is designated as, or can be, a
15 hydrogen, the exact chemical nature of a substituent which is other than hydrogen at that position, e.g., a hydrocarbyl radical or a halogen, hydroxy, amino and the like functional group, is not critical so long as it does not adversely affect the overall activity and/or
20 synthesis procedure.

The chemical reactions described above are generally disclosed in terms of their broadest application to the preparation of the compounds of this invention. Either the reactions can be successfully
25 performed by conventional modifications known to those skilled in the art, e.g., by appropriate protection of interfering groups, by changing to alternative conventional reagents, by routine modification of reaction conditions, and the like, or other reactions
30 disclosed herein or otherwise conventional, will be applicable to the preparation of the corresponding compounds of this invention. In all preparative methods, all starting materials are known or readily preparable from known starting materials.

35 The following preferred specific embodiments are, therefore, to be construed as merely illustrative

and not limitative of the remainder of the disclosure in any way whatsoever.

The following examples are provided to illustrate the present invention and are not intended to limit the scope thereof. Those skilled in the art will readily understand that known variations of the conditions and processes of the following preparative procedures can be used to prepare these compounds. All temperatures expressed are in degrees centigrade. Within the foregoing synthetic description and examples which follow, abbreviations have the following meanings:

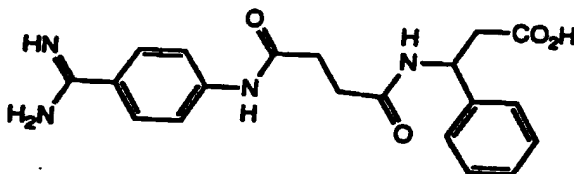
	CHCl ₃	=	chloroform
	DMF	=	dimethylformamide
15	DMSO	=	dimethylsulfoxide
	g	=	gram
	MeOH	=	methanol
	min	=	minute
	h	=	hour
20	mol	=	mole
	mmol	=	mmole
	MW	=	molecular weight
	TLC	=	thin layer chromatography
	NMM	=	N-methylmorpholine
25	RPHPLC	=	Reverse Phase High Pressure Liquid Chromatography
	TDA-1	=	Tris[2-(2methoxyethoxy)ethyl]amine
	PTC	=	Phase Transfer Catalysis

Example 1

3-[[4-[[4-(aminoiminomethyl)phenyl]amino]-
1,4-dioxobutyl]amino]3-phenylpropionic acid.

5

10

Step 1 Preparation of 4-[[4-

15 (aminoiminomethyl)phenyl]-amino]-4-oxobutanoic acid.

4-Aminobenzamidine di-HCl (25 g, 120 mmol), which is commercially available particularly from Aldrich, was added to dry DMF (100 ml). To this solution dry pyridine (100 ml) and succinic anhydride

20 (12 g, 120 mmol) followed by dimethylaminopyridine (DMAP 1.5 g 0.012 mmol) were added. The product precipitated after heating for 1/2 h at 100°C. The product was filtered, washed with water, acetonitrile and ether. The light solid was suspended in dioxane, 4N HCl in
25 dioxane (100 ml) was added and the suspension was stirred for 1 h, filtered and dried in a desiccator to give 28 g, 88% of 4-[[4-(aminoiminomethyl)phenyl]amino]-4-oxo-butanoic acid as a white yellow solid which decomposes between 270° and 290°C.

30 Step 2 Preparation of D,L-3-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-3-phenylpropionic acid.

4-[[4-(Aminoiminomethyl)phenyl]-amino]-4-oxobutanoic acid hydrochloride prepared in Step
35 1 (1 g, 3.7 mmol) was added to dry DMF (35 ml) followed by N-methylmorpholine (0.39 g, 1 eq.) and isobutyl chloroformate (0.53 g, 3.9 mmol) at 25°C. The mixture

was stirred for 5 min. D,L-3-Amino-3-phenylpropionic acid (0.67 g, 4.05 mmol) was added followed by diisopropylethylamine (0.68 mL; 3.9 mmol) and a catalytic amount of dimethylaminopyridine. After 1 hr, the solvent was removed under reduced pressure and the product was purified by reverse phase chromatography (0.05% TFA water/acetonitrile) and lyophilized to give 340 mg of white solid: ^1H NMR (d_6 -DMSO) δ 2.45 (m, 2H), 2.6 (m, 2H), 2.7 (d, 2H, $J = 7$ Hz), 4.2 (dd, 1H, $J = 7$ Hz and 8 Hz), 7.3 (m, 4H), 7.8 (s, 4H), 8.45 (d, 1H, $J = 8$ Hz), 9.0 (bs, 2H), 9.2 (bs, 2H), 10.4 (s, 1H); MS (FAB) m/z 383.2 (MH $^+$).

Elemental Analysis

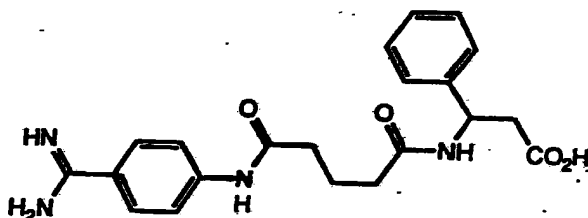
Required for

15	$\text{C}_{20}\text{H}_{22}\text{N}_4\text{O}_4 \cdot \text{F}_3\text{C}_2\text{O}_2\text{H} \cdot \text{H}_2\text{O}$:	C 51.36	H 4.90	N 10.90
	Found:	C 51.67	H 4.74	N 10.72

Example 2

20 3-[[4-[[4-aminoiminomethyl)phenyl]amino]-1,5-dioxopentyl]amino]-3-phenylpropionic acid.

25



30

Step 1 Preparation of 4-[[4-(aminoiminomethyl)phenyl]-amino]-5-oxopentanoic acid.

4-Aminobenzamidine di-HCl (1 g, 4.8 mmol) was added to dry DMF (20 mL). To this solution dry pyridine (5 mL) and glutaric anhydride (0.68 g, 5.3 mmol) followed by 10 mg dimethylaminopyridine (DMAP) were

added. The product started to precipitate after heating for 1/2 h at 100°C. Heating was continued for 2 hr and water (25 mL) was added after cooling to room temperature. An abundant precipitate was filtered and

- 5 dried in a desiccator to give 0.8 g, 50% of product as a white solid: ¹H NMR (d₆-DMSO) δ 1.95 (m, 2H), 2.4 (m, 2H), 2.5 (m, 2H), 7.85 (s, 4H), 9.05 (bs, 2H), 9.25 (bs, 2H), 10.4 (s, 1H), MS(FAB) m/z 250.1 (MH⁺).

Step 2 Preparation of D,L-3-[[4-[[4-

- 10 (aminoiminomethyl)-phenyl]amino]1,5-dioxopentyl]amino]-3-phenylpropionic acid.

- An aliquot of 4-[[4-(aminoiminomethyl)phenyl]-amino]-5-oxopentanoic acid prepared in Step 1 (1 g, 3.5 mmol) was dissolved in dry DMF (35 ml) and N-
- 15 methylmorpholine (0.39 g, 1 eq.) and isobutyl chloroformate (0.5 g) were added to the mixture cooled to 0°C. The mixture was stirred for 5 min. D,L-3-Amino-3-phenylpropionic acid (0.58 g) was added followed by a catalytic amount of dimethylaminopyridine. After
- 20 1 h, the solvent was removed under reduced pressure and the product was purified by reverse phase chromatography (0.056% TFA water/acetonitrile) to give 440 mg of white fluffy solid: ¹H NMR (d₆-DMSO) δ 1.80 (m, 2H), 2.18 (t, 2H, J = 7 Hz), 2.4 (t, 2H, J = 7 Hz), 2.65 (d, 2H, J = 7
- 25 Hz), 4.2 (dd, 1H, J = 7 Hz and 8 Hz), 7.3 (m, 4H), 7.8 (s, 4H), 8.35 (d, 1H, J = 8 Hz), 8.95 (bs, 2H), 9.18 (bs, 2H), 10.34 (s, 1H); MS (FAB) m/z 397.2 (MH⁺), 351, 232.

Elemental Analysis

- 30 Required for

C₂₁H₂₄N₄O₄·F₃C₂O₂H·H₂O: C 52.27 H 5.15 N 10.60

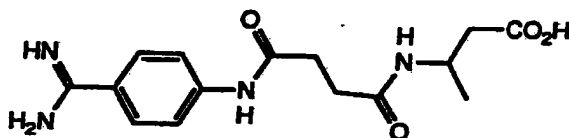
Found: C 52.19 H 5.12 N 10.38

Example 3

3-[[4-[[4-(aminoiminomethyl)phenyl]amino]-
1,4-dioxobutyl]amino]-butanoic acid.

5

10



An aliquot of 4-[[4-(aminoiminomethyl)phenyl]-
15 amino]-4-oxobutanoic acid prepared in Example 1, Step 1
(2 g) was added to dry DMF (65 ml) followed by N-
methylmorpholine (0.75 g, 1 eq.) and isobutyl
chloroformate (1 g) at 25°C. The mixture was stirred
for 5 min. 3-Aminobutyric acid (1.1 g, 1.1 eq.) was
20 added followed by triethylamine (1.5 g, 1.3 eq.) and
dimethylaminopyridine. After 1 h, the solvent was
removed under reduced pressure and the product was
purified by reverse phase chromatography (0.05% TFA
water/acetonitrile) to give 750 mg of white solid: ¹H
25 NMR (d₆-DMSO) δ 1.06 (d, 3H, J = 7 Hz), 2.2-2.6 (m, 6H),
4.05 (m, 1H), 7.8 (m, 4H), 7.85 (d, 1H, J = 8 Hz), 9.05
(bs, 2H), 9.15 (bs, 2H), 10.4 (s, 1H); MS (FAB) m/z
321.1 (MH⁺), 236.

Elemental Analysis

30 Required for

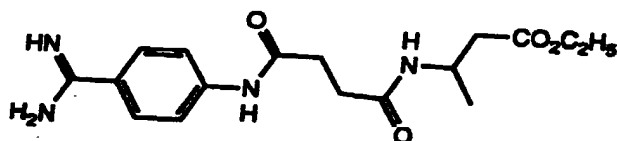
C ₁₅ H ₂₀ N ₄ O ₄ ·F ₃ C ₂ O ₂ H·0.75H ₂ O:	C 45.66	H 4.90	N 12.52
Found:	C 45.54	H 4.27	N 12.41

Example 4

Ethyl-3-[[4-[[4-(aminoiminomethyl)phenyl]-
amino]-1,4-dioxobutyl]amino]-butanoate.

5

10



4-[[4-(aminoiminomethyl)phenyl]-
amino]-4-oxobutanoic acid hydrochloride prepared in
Example 1, Step 1 (5 g, 18 mmol) was added to dry DMF
(100 ml) followed by N-methylmorpholine (2.2 g, 22 mmol)
and isobutyl chloroformate (2.8 g, 22 mmol) at 25°C.
The mixture was stirred for 5 min. Ethyl 3-amino
butyrate (2.5 g, 22 mmol) was added followed by
dimethylaminopyridine. After 1 hr, the solvent was
removed under reduced pressure and the product was
purified by reverse phase chromatography
(0.05% TFA water/acetonitrile) to give 4.4 g of white
solid: ¹H NMR (d₆-DMSO) δ 1.06 (d, 3H, J = 7 Hz), 2.3-
2.6 (m, 6H), 4.05 (m, 3H), 7.8 (s, 4H), 7.9 (d, 1H, J =
8 Hz), 9.1 (bs, 2H), 9.2 (bs, 2H), 10.4 (s, 1H); MS
(FAB) m/z 349.2 (MH⁺), 321, 218.

Elemental Analysis

Required for

C ₁₇ H ₂₄ N ₄ O ₄ ·F ₃ C ₂ O ₂ H:	C 49.35	H 5.44	N 12.11
Found:	C 49.18	H 5.44	N 11.98

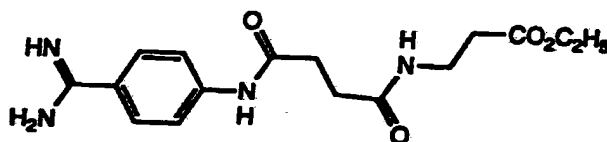
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Example 5

Ethyl-3-[[4-[[4-(aminoimino-
methyl)phenyl]amino]-1,4-dioxobutyl]amino]propanoate.

5

10



An aliquot of 4-[[4-(aminoiminomethyl)phenyl]-
amino]-4-oxobutanoic acid hydrochloride prepared in
Example 1, Step 1 (1.36 g) was added to dry DMF (50 ml)
followed by N-methylmorpholine (0.6 mL) and isobutyl
chloroformate (0.65 mL) at 0°C under nitrogen
atmosphere. The mixture was stirred for 5 min, then
0.45 g β -alanine ethyl ester hydrochloride was added
followed by 0.6 mL of N-methylmorpholine. After 4 hr,
the solvent was removed under reduced pressure and the
product was purified by reverse phase chromatography
(0.05% TFA water/acetonitrile) to give 4.4 g of white
solid: ^1H NMR (d_6 -DMSO) δ 1.2 (t, 3H, $J = 7$ Hz), 2.45
(m, 4H), 2.6 (m, 2H), 3.25 (m, 2H), 4.05 (q, 2H, $J = 7$
Hz), 7.8 (s, 4H), 8.0 (m, 1H), 8.85 (bs, 2H), 9.18 (bs,
2H), 10.4 (s, 1H); MS (ES) m/z 335.1 (MH $^+$).

Elemental Analysis

30 Required for

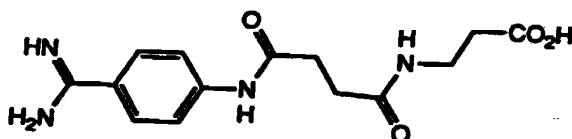
$\text{C}_{16}\text{H}_{22}\text{N}_4\text{O}_4 \cdot 1.5\text{F}_3\text{C}_2\text{O}_2\text{H} \cdot 0.5\text{H}_2\text{O}$	C 43.21	H 4.53	N 11.20
Found	C 43.56	H 4.70	N 11.07

Example 6

3-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]propionic acid.

5

10



A portion of ethyl-3-[[4-[[4-aminoiminomethyl)-phenyl]amino]-1,4-dioxobutyl]amino]propanoate (300 mg) was dissolved in 10 mL water. Sodium hydroxide (2N) was added until the pH reached 10. The reaction mixture was allowed to stir at 25°C for 30 min during which time a precipitate appeared. The mixture was acidified with HCl to pH 5. The precipitate was filtered, washed with water and diethyl ether and purified by RPHPLC (acetonitrile/water) to give 50 mg of a white powder: ¹H NMR (d₆-DMSO) δ 2.4 (m, 4H), 2.6 (m, 2H), 3.25 (m, 2H), 7.8 (s, 4H), 8.0 (t, 1H, J = 7 Hz), 8.80 (bs, 2H), 9.18 (bs, 2H), 10.4 (s, 1H); MS (ES) m/z 307.1 (MH⁺).

Elemental Analysis

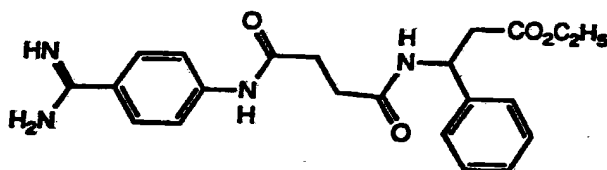
Required for

 $C_{14}H_{18}N_4O_4 \cdot F_3C_2O_2H \cdot 0.5H_2O$: C 44.86 H 4.47 N 13.08

Found: C 45.03 H 4.55 N 12.99

Example 7

Preparation of ethyl-3-[[4-[[4-(aminoiminomethyl)-phenyl] amino]-1,4-dioxobutyl]amino]-3-phenylpropanoate.



In a round bottom flask under a static atmosphere of dry nitrogen were mixed 1.3 g of D,L-3-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-3-phenylpropanoic acid prepared as described in Example 1, Step 2, 200 mL absolute ethanol and 10 mL 4 N HCl in dioxane. The reaction mixture was stirred at 25°C for 16 hr. The volatiles were removed in vacuo and the remaining white solid was purified by RPHPLC (.05% TFA water/acetonitrile gradient; 95/5 to 30/70 over 30 min) to provide 0.62 g of the desired ester as a white solid: ¹H NMR (d₆-DMSO) δ 1.1 (t, 3 H, J = 7 Hz), 2.45 (m, 2H), 2.6 (m, 2H), 2.75 (d, 2H, J = 7 Hz), 4.0 (q, 2H, J = 7 Hz), 4.2 (dd, 1H, J = 7 Hz and 8 Hz), 7.3 (m, 4H), 7.8 (s, 4H), 8.45 (d, 1H, J = 8 Hz), 9.05 (bs, 2H), 9.2 (bs, 2H), 10.4 (s, 1H); MS (FAB) m/z 411.2 (MH⁺), 135.2.

Elemental Analysis

Required for

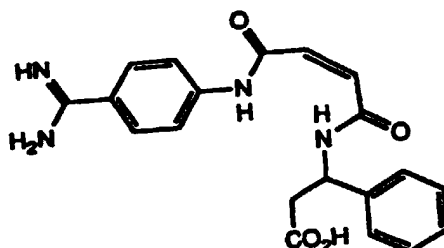
C ₂₂ H ₂₆ N ₄ O ₄ ·F ₃ O ₂ H·H ₂ O:	C 53.13	H 5.41	N 10.24
Found:	C 53.13	H 5.39	N 10.33

Example 8

3-[[4-[[4-(aminoiminomethyl)phenylamino]-1,4-dioxobuten-(Z)-yl]amino]-phenylpropanoic acid.

5

10



The compound was prepared from maleic
15 anhydride, aminobenzamidine and 3-amino phenylpropionic
acid in a manner similar to that described in Example 1.
¹H NMR (d₆-DMSO) δ, 2.45 (m, 2H), 3.6 (m, 2H), 7.05 (m,
2H), 7.85 (m, 4H), 8.6 (m, 1H), 8.9 (bs, 2H), 9.1 (d,
1H, J = 7 Hz), 9.25 (bs, 2H), 10.85 (s, 1H); MS (ES) m/z
20 381.2 (MH⁺), 216.

Elemental Analysis

Required for

C₂₀H₂₀N₄O₄·F₃C₂O₂H·1.5H₂O: C 50.67 H 4.64 N 10.74

Found: C 50.28 H 4.15 N 10.63

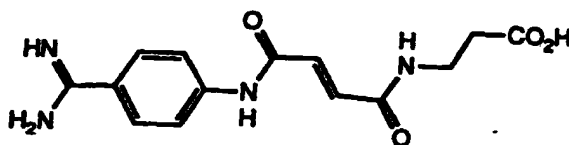
25

Example 9

3-[[4-[[4-(aminoiminomethyl)phenylamino]-1,4-dioxobuten-(E)-yl]amino]-propanoic acid.

30

35



Step 1 Preparation of 4-[[4-(aminoiminomethyl)phenyl]amino]-4-oxobuten-(E)-oic acid.

In a round bottomed flask under a static atmosphere of dry nitrogen were mixed 1.4 g of monoethyl fumarate, 1.36 g of isobutyl chloroformate and 1.01 g N-methylmorpholine in 100 mL DMF. 4-aminobenzamidine dihydrochloride (2.06 g) and 2.02 g N-methylmorpholine were added at room temperature and the reaction mixture was stirred at 25°C for 30 min. Water and sodium hydroxide were added to pH 10 and after one hour of stirring the reaction was neutralized to pH 7 to precipitate the zwitterion. Filtration provided 1 g of the desired compound as a white solid : ¹H NMR (d₆-DMSO) δ 1.1 (t, 3 H, J = 7 Hz), 2.45 (m, 2H), 2.6 (m, 2H), 2.75 (d, 2H, J = 7 Hz), 4.0 (q, 2H, J = 7 Hz), 4.2 (dd, 1H, J = 7 Hz and 8 Hz), 7.3 (m, 4H), 7.8 (s, 4H), 8.45 (d, 1H, J = 8 Hz), 9.05 (bs, 2H), 9.2 (bs, 2H), 10.4 (s, 1H).

Step 2 Preparation of ethyl-3-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobuten-(E)-yl]amino]-propanoate.

4-[[4-(aminoiminomethyl)phenyl] amino]-4-oxobuten-(E)-oic acid prepared in Example 9, Step 1 (1.35 g) was added to dry DMF (50 mL) followed by N-methylmorpholine (0.55 mL) and isobutyl chloroformate (0.65 mL) under a nitrogen atmosphere. The mixture was stirred for 5 min, and then 0.75 g of β-alanine ethyl ester hydrochloride was added followed by 0.55 mL of N-methylmorpholine. After 2 hr, the solvents were removed under reduced pressure and the product was purified by RPHPLC(water/acetonitrile) to give 700 mg of white solid.

Step 3 3-[[4-[[4-(Aminoiminomethyl)phenyl]amino]-1,4-dioxobuten-(E)-yl]amino]-propanoic acid.

A portion of ethyl 3-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobuten-(Z)-yl]amino]-propanoate (150 mg of the trifluoroacetate

salt) prepared as in Example 9, step 2 was dissolved in 10 mL water and 10 mL acetonitrile and 5 drops of 50% sodium hydroxide were added. The reaction mixture was allowed to stir at 25°C for 1 hr, and was then acidified to pH 5. The precipitate was collected by filtration, washed with acetonitrile, water, and diethylether to give 120 mg of a white powder which was lyophilized from HCl to give the hydrochloride salt: ¹H NMR (d₆-DMSO) δ 2.45 (m, 2H), 3.6 (m, 2H), 7.0 (m, 2H), 7.85 (m, 4H), 8.63 (m, 1H), 8.85 (bs, 2H), 9.20 (bs, 2H), 10.9 (s, 1H); MS (ES) m/z 333.1 (MH⁺).

Elemental Analysis

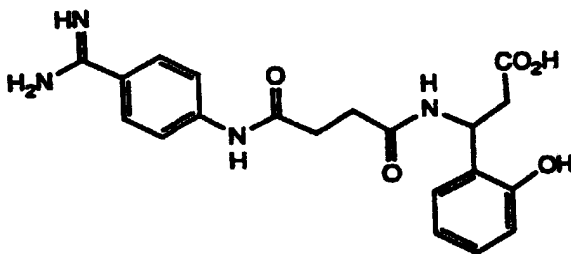
Required for

C₁₄H₁₇N₄O₄·1.5 F₃C₂O₂H·0.5H₂O: C 41.39 H 3.98 N 11.36

Found: C 41.55 H 3.83 N 11.72

Example 10

Preparation of 3-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-3-(2-hydroxyphenyl)propanoic acid.



In a flask under nitrogen, the succinyl derivative prepared in Example 1, Step 1 (5.7 g) was activated with isobutyl chloroformate (2.9 g) and coupled with 4.2 g of 3-amino-3,4-dihydro-2-oxo-2H-1-benzopyran hydrochloride in a manner similar to Step 2, Example 1. The reaction mixture was worked up as usual and the product purified by RPHPLC to give 2.9 g of 3-

[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-3-(2-hydroxyphenyl)propanoic acid as a white powder : ^1H NMR (d_6 -DMSO) δ 2.5 (m, 6H), 4 (m, 1H), 6.7 (t, 1H, $J = 7.5$ Hz), 6.8 (d, 1H, $J = 7.5$ Hz), 7.05 (t, 1H, $J = 7.5$ Hz), 7.2 (d, 1H, $J = 7.5$ Hz), 7.8 (s, 4H), 8.25 (d, 1H, $J = 8$ Hz), 8.95 (bs, 2H), 9.2 (bs, 2H), 10.4 (s, 1H); MS (FAB) m/z 399.1 (MH^+), 235.

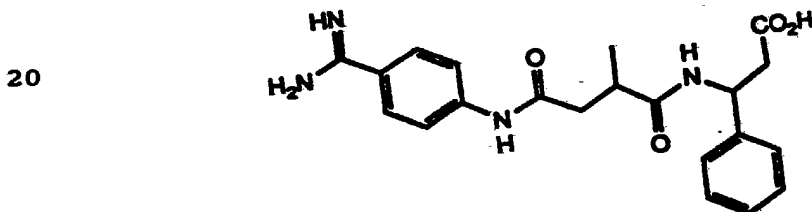
Elemental Analysis

Required for

10 $\text{C}_{20}\text{H}_{22}\text{N}_4\text{O}_5 \cdot \text{F}_3\text{C}_2\text{O}_2\text{H} \cdot 2\text{H}_2\text{O}$: C 48.25 H 4.95 N 10.25
Found: C 48.72 H 5.56 N 10.21

Example 11

15 3-[[4-[[4-(aminoiminomethyl)phenyl]amino]-2(3)-methyl-1,4-dioxobutyl]amino]-3-phenylpropionic acid



25

Step 1 Preparation of 4-[[4-(aminoiminomethyl)phenyl]amino]-2(3)-methyl-4-oxobutanoic acid.

A procedure similar to Step 1 of Example 1 using 5 g of aminobenzamidine di-HCl and 2.85 g methyl succinic anhydride was used. The product was filtered, washed with water, acetonitrile and ether. The white solid (4.5 g) was suspended in dioxane, 4N HCl in dioxane (100 ml) was added and the suspension was stirred for 1 h, filtered and dried.

35

Step 2 Preparation of D,L-3-[[4-[[4-(aminoiminomethyl)phenyl]amino]-2(3)-methyl-1,4-dioxobutyl]amino]-3-phenylpropionic acid.

N-2(3)Methyl-4-succinylamidobenzamidine

- 5 hydrochloride prepared in Step 1 (1.68 g, 5.8 mmol) was activated with isobutyl chloroformate (0.78 mL, 6 mmol) and coupled with D,L-3-amino-3-phenylpropionic acid (0.96 g, 5.8 mmol) in a manner similar to Example 12. After usual work up, the reaction mixture was purified
- 10 by reverse phase chromatography (0.05% TFA water/acetonitrile). Two peaks (A and B) were isolated and lyophilized. Peak A gave 340 mg of yellow solid : ¹H NMR (d₆-DMSO) δ 1.0 (m, 3H), 2.4-2.6 (m, 4H), 2.8 (m, 2H), 4.1 (m, 1H), 7.15 (m, 4H), 7.7 (s, 4H), 8.45 (m,
- 15 1H), 9.0 (bs, 2H), 9.2 (bs, 2H), 10.4 (d, 1H, 8 Hz); MS (FAB) m/z 397.3 (MH⁺).

Elemental Analysis,

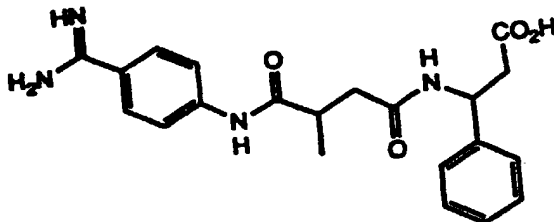
Required for

C₂₃H₂₇N₄O₇·F₃C₂O₂H·1.5H₂O: C 51.40 H 5.25 N 10.42

20 Found: C 51.69 H 4.86 N 10.38

Example 12

- 3-[[4-[[4-(aminoiminomethyl)phenyl]amino]-
- 25 3(2)-methyl-1,4-dioxobutyl]amino]-3-phenylpropionic acid

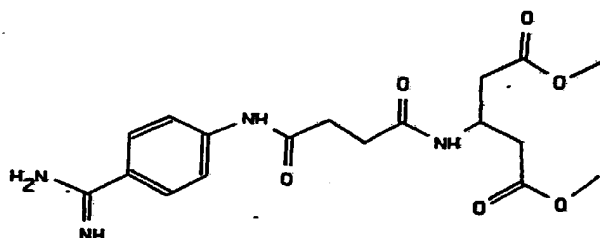


- 35 N-2(3)Methyl-4-succinylamidobenzamidine hydrochloride prepared in Step 1 Example 11(1.68 g, 5.8 mmol) was activated with isobutyl chloroformate (0.78

mL, 6 mmol) and coupled with D,L-3-amino-3-phenylpropionic acid (0.96 g, 5.8 mmol) in a manner similar to Step 2 of Example 11. After usual work up, the reaction mixture was purified by reverse phase chromatography (0.05% TFA water/acetonitrile). Two peaks (A and B) were isolated and lyophilized. Peak A was described in Example 11. Step 2. Peak B isolated from the crude reaction mixture of Example 11, Step 2 gave 540 mg of white solid: ^1H NMR (d_6 -DMSO) δ 1.05 (m, 3H) 2.2-2.6 (m, 5H), 2.85 (m, 1H), 5.15 (m, 1H), 7.2 (m, 1H), 7.25 (s, 4H), 7.8 (s, 4H), 8.45 (m, 1H), 8.75 (bs, 2H), 9.15 (bs, 2H), 10.4 (bs, 1H); MS (FAB) m/z 397.3 (MH $^+$).

Example 13

Dimethyl 3-[[4-[[4-(aminoiminomethyl)phenyl]-amino]-1,4-dioxobutyl]amino]pentanedioate.



Step 1 Preparation of dimethyl-3-aminoglutarate.

Dimethyl-3-oxoglutarate (10 g, 57 mmol) was added to methanol (225 ml) followed by ammonium formate (36 g, 570 mmol) and NaBH_3CN (3.7 g, 57 mmol) at 25°C. After 24 h, the methanol was removed in vacuo to leave a white mass. Methylene chloride was added and the mixture filtered. The methylene chloride was evaporated resulting in an oil which was dissolved in 1N HCl (200 ml) and extracted with ether (100 ml). The ether layer was discarded and the aqueous layer was made basic using

solid K_2CO_3 . The product was extracted into methylene chloride, dried over Na_2SO_4 , and evaporated to give dimethyl-3-aminoglutarate (7.5 g). 1H NMR (d_6 -DMSO) δ 1.76 (bs, 2H), 2.45 (dd, 4H, $J=8.1$ Hz, 16.6 Hz), 3.69 (s, 6H), 5.45 (m, 1H); MS (FAB) m/z 176.0 (MH^+).

Step 2 Preparation of dimethyl 3-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]pentanedioate.

4-[[4-(aminoiminomethyl)phenyl]-amino]-4-oxobutanoic acid hydrochloride prepared in Example 1, Step 1 (4.6 g, 17 mmol) was added to dry DMF (225 ml) followed by N-methylmorpholine (1.2 g, 17 mmol) and isobutyl chloroformate (2.3 g, 17 mmol) at 25°C. The mixture was stirred for 5 min. Dimethyl-3-aminoglutarate (3.0 g, 17 mmol) was added followed by dimethylaminopyridine. After 1 hr, the solvent was removed under reduced pressure and the product purified by reverse phase chromatography (0.05% TFA water/acetonitrile) to result in 3.5 g of a white solid: 1H NMR (d_6 -DMSO) δ 2.37 (t, 2H, $J=7.3$ Hz), 2.55 (m, 2H), 2.57 (t, 2H, $J=7.1$ Hz), 3.57 (s, 6H), 4.35 (m, 1H), 7.79 (s, 4H), 7.99 (d, 1H, $J=8.1$ Hz), 9.1 (bs, 2H), 9.19 (bs, 2H), 10.42 (s, 1H); MS (FAB) m/z 393.2 (MH^+).

Elemental Analysis

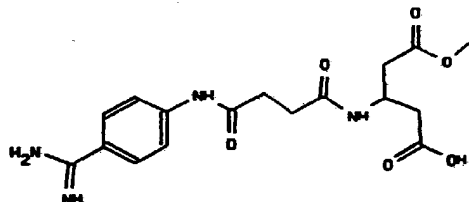
25 Required for

$C_{18}H_{24}N_4O_6 \cdot F_3C_2O_2H \cdot H_2O$: C 47.42 H 4.91 N 11.14

Found: C 47.12 H 4.97 N 10.99

Example 14

3-[[4-[[4-(aminoiminomethyl)phenyl]-amino]-
1,4-dioxobutyl]amino]pentanedioic acid, monomethylester.



Dimethyl 3-[[4-[[4-(aminoiminomethyl)phenyl]-
amino]-1,4-dioxobutyl]amino]pentanedioate prepared in
Example 13, Step 2 (700 mg) was added to
water/acetonitrile (20 ml) followed by lithium hydroxide
(100 mg) at 25°C. The mixture was stirred for 30 min.
The course of the reaction was monitored by RPHPLC.
After a satisfactory quantity of monoester was formed
the reaction was neutralized with TFA and purified by
RPHPLC (0.05% TFA water/acetonitrile) to result in 460
mg of a white solid: ¹H NMR (d₆-DMSO) δ 2.39 (t, 2H, J
= 7.3 Hz), 2.55 (m, 2H), 2.57 (t, 2H, J = 7.1 Hz), 3.57
(s, 3H), 4.32 (m, 1H), 7.78 (s, 4H), 7.99 (d, 1H, J =
8.1 Hz), 8.92 (bs, 2H), 9.16 (bs, 2H), 10.39 (s, 1H);
MS (FAB) m/z 379.2 (MH⁺).

Elemental Analysis

Required for

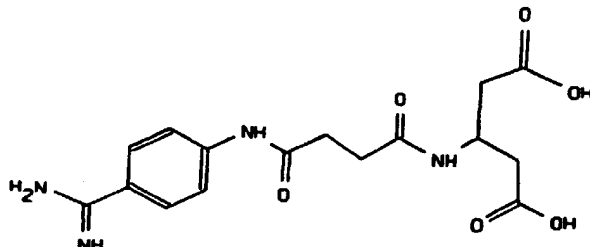
30	C ₁₇ H ₂₂ N ₄ O ₆ · F ₃ C ₂ O ₂ H · H ₂ O:	C 45.92	H 4.63	N 11.28
	Found:	C 45.88	H 4.34	N 10.69

Example 15

3-[[4-[[4-(aminoiminomethyl)phenyl]-amino]-1,4-dioxobutyl]amino]pentanedioic acid.

5

10



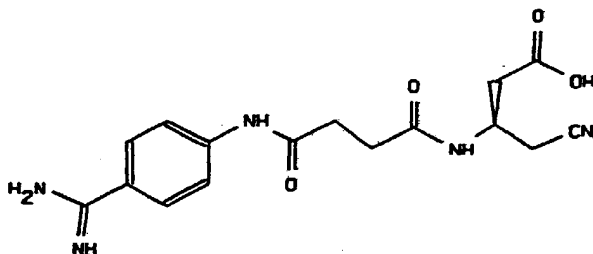
Dimethyl 3-[[4-[[4-(aminoiminomethyl)phenyl]-amino]-1,4-dioxobutyl]amino]pentanedioate prepared in Example 13, Step 2 (700 mg) was added to water/acetonitrile, (20 ml) followed by lithium hydroxide (100 mg) at 25°C. The mixture was stirred for 30 min. The course of the reaction was monitored by RPHPLC. After satisfactory monoester was formed the reaction was neutralized with TFA and purified by RPHPLC (.05% TFA water/acetonitrile) to result in 620 mg of a white solid: ¹H NMR (d₆-DMSO) δ 2.38 (t, 2H, J = 7.3 Hz), 2.44 (d, 2H, J = 6.4 Hz), 2.56 (t, 2H, J = 7.3 Hz), 4.32 (m, 1H), 7.78 (s, 4H), 7.99 (d, 1H, J = 8.1 Hz), 8.92 (bs, 2H), 9.16 (bs, 2H), 10.39 (s, 1H); MS (FAB) m/z 365.2 (MH⁺).

Elemental Analysis
Required for

30 C₁₆ H₂₀ N₄ O₆ · F₃C₂O₂H · H₂O: C 43.54 H 4.64 N 11.13
Found: C 43.40 H 4.52 N 11.18

Example 16

3-(R)-[[4-[[4-(aminoiminomethyl)phenyl]-
amino]-1,4-dioxobutyl]amino]-4-cyanobutanoic acid.



Step 1 Preparation of benzyl-3-amino-4-cyano
butyrate.

Benzyl-3-N-t-Boc-amino-4-hydroxy-(3S)-butyrate (20 g, 64.7 mmol) was dissolved in 200 ml of methylene chloride followed by triethylamine (9.8 g, 97 mmol) and cooled to 0°C. Methanesulfonyl chloride (9.6 g, 84 mmol) was added and the solution was stirred for 2-3 hr. After this time more methylene chloride (100 ml) was added and the solution was washed with water, and dried over MgSO₄ to give 27 g of the mesylate after removal of the solvent. ¹H NMR (d6-DMSO) δ 1.45 (s, 9H), 2.71 (d, 2H, J = 6 Hz), 2.95 (s, 3H), 4.37 (bs, H), 4.7 (bs, 2H), 5.15 (s, 2H), 7.37 (bs, 5H).

The mesylate (27 g, 64 mmol) isolated above was added to dry DMF followed by KCN, 18-crown-6, catalytic DMAP and heated at 70° C for 2-3 h. After complete reaction, water was added and the product was extracted with ether (2x150 ml). The ether extracts were washed with water, then dried over MgSO₄, and the solvent evaporated to give benzyl-3-N-t-Boc-amino-4-cyano-(3S)-butyrate (22g).

The crude benzyl-3-N-t-Boc-amino-4-cyano-(3S)-butyrate was dissolved in dioxane (100 ml), and then to this solution 4N HCl in dioxane was added. After 6h,

the dioxane was removed in vacuo to leave an oil which was dissolved in water (200 ml), and extracted with ether (100 ml). The ether layer was discarded and the aqueous layer was made basic using solid K_2CO_3 . The product was extracted into methylene chloride, dried over Na_2SO_4 , and evaporated to give benzyl-3-amino-4-cyano-(3S)-butyrate (8 g). 1H NMR (d_6 -DMSO) δ 1.6 (bs, 2H), 2.5-2.7 (m, 4H), 3.5 (m, 1H), 5.16 (s, 2H), 7.36 (bs, 5H); H), 4.05 (m, 3H), 7.8 (s, 4H); MS (FAB) m/z 219.0 (MH^+).

Step 2 Preparation of 3-(R)-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-4-cyanobutanoic acid.

4-[[4-(aminoiminomethyl)phenyl]amino]-4-oxobutanoic acid hydrochloride prepared in Example 1, Step 1 (5.1 g, 18.9 mmol) was added to dry DMF (250 ml), followed by N-methylmorpholine (1.8 g, 18.9 mmol) and isobutyl chloroformate (2.7 g, 18.9 mmol) at 25°C. The mixture was stirred for 5 min. Benzyl-3-amino-4-cyano butyrate (3.0 g, 18.9 mmol; from Step 1) was added followed by dimethylaminopyridine. After 1 h, the solvent was removed under reduced pressure and the product purified by RPHPLC (0.05% TFAwater/acetonitrile) to result in 3.5 g of a white solid. A portion of this material was then subjected to saponification conditions as previously described and purified by reverse phase chromatography (water/acetonitrile) to result in 425 mg of a white solid: 1H NMR (d_6 -DMSO) δ 2.44 (m, 2H), 2.5 (m, 2H), 2.60 (m, 2H), 2.74 (dd, 2H, $J = 6.9, 11.8$ Hz), 4.25 (m, 1H), 7.78 (s, 4H), 8.26 (d, 1H, $J = 7.7$ Hz), 8.89 (bs, 2H), 9.14 (bs, 2H), 10.40 (s, 1H); MS (FAB) m/z 346.1 (MH^+).

Elemental Analysis

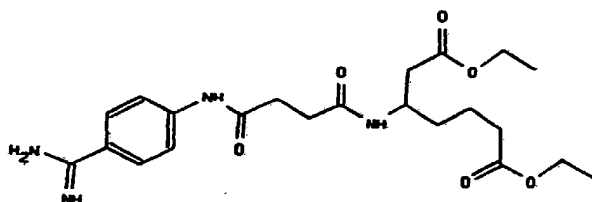
Required for

35	$C_{16}H_{19}N_5O_4 \cdot F_3C_2O_2H \cdot H_2O$:	C 46.15	H 4.48	N 14.95
	Found:	C 46.15	H 4.28	N 14.76

Example 17

(±)-Diethyl-3-[[4-[[4-(aminoiminomethyl)phenyl]amino]-4-dioxobutyl]amino]heptanedioate.

10



15 Step 1 Preparation of diethyl-3-aminopimaleate. Diethyl-3-oxopimaleate (10 g, 43 mmol) was added to methanol (225 ml), followed by ammonium formate (27.4 g, 430 mmol) and NaBH_3CN (2.7 g, 43 mmol) at 25°C. After 24 h, the methanol was removed in vacuo to leave a white mass. Methylene chloride was added and the mixture filtered. The methylene chloride was evaporated resulting in an oil which was dissolved in 1N HCl (200 ml) and extracted with ether (100 ml). The ether layer was discarded, and the aqueous layer was made basic using solid K_2CO_3 . The product was extracted into methylene chloride, dried over Na_2SO_4 , and evaporated to give diethyl-3-aminopimaleate (7.5 g). ^1H NMR ($\text{d}_6\text{-DMSO}$) δ 1.25 (t, 3H, $J = 7$ Hz), 1.26 (t, 3H, $J = 8$ Hz), 1.45 (m, 2H), 1.7 (m, 2H), 2.01 (bs, 2H), 2.45 (m, 2H), 3.2 (m, 1H), 4.13 (q, 4H, $J = 8$ Hz); MS (FAB) m/z 132.1 (MH^+) 186.2.

Step 2 Preparation of diethyl 3-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]heptanedioate. 4-[[4-(Aminoiminomethyl)phenyl]amino]-4-oxobutanoic acid hydrochloride prepared in Example 1, Step 1 (5.0 g, 18.5 mmol) was added to dry DMF (250 ml)

followed by N-methylmorpholine (2.2 g, 18.5 mmol) and isobutyl chloroformate (2.7 g, 18.5 mmol) at 25°C. The mixture was stirred for 5 min. Diethyl-3-aminopimaleate (4.25 g, 18.5 mmol; from Step 1) was added followed by
 5 dimethylaminopyridine. After 1 hr, the solvent was removed under reduced pressure, and the product purified by RPHPLC (0.05% TFA water/acetonitrile) to result in 4.1 g of a white solid: ¹H NMR (d₆-DMSO) δ 1.15 (t, 3H, J = 7.3 Hz), 1.16 (t, 3H, J = 8 Hz), 1.4 (m, 2H), 2.50
 10 (t, 2H, J = 7.1 Hz), 2.49 (m, 4H), 2.58 (t, 2H, J = 7.1 Hz), 4.04 (m, 5H), 7.78 (s, 4H), 7.79 (d, 1H, J = 12.4 Hz), 8.95 (bs, 2H), 9.15 (bs, 2H), 10.40 (s, 1H), MS (FAB) m/z 449.0 (MH⁺).

Elemental Analysis

15 Required for

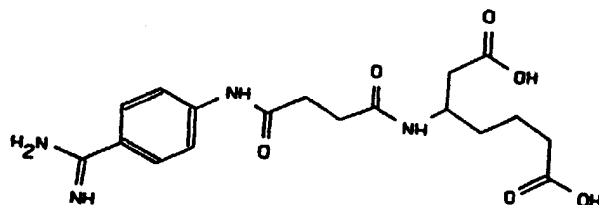
C ₂₂ H ₃₂ N ₄ O ₆ · F ₃ C ₂ O ₂ H · H ₂ O:	C 50.44	H 5.95	N 9.80
Found:	C 50.33	H 6.02	N 9.67

Example 18

20

3-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]heptanedioic acid.

25



30

Diethyl-3-[[4-[[4-(aminoiminomethyl)phenyl]-amino]-1,4-dioxobutyl]amino]heptanedioate prepared in Example 17, Step 2 (700 mg) was added to
 35 water/acetonitrile (20 ml) followed by lithium hydroxide (100 mg) at 25°C. The mixture was stirred for 30 min. The course of the reaction was monitored by RPHPLC.

After satisfactory monoester was formed the reaction was neutralized with TFA and purified by reverse phase chromatography (0.05% TFA water/acetonitrile) to result in 550 mg of a white solid: ^1H NMR (d_6 -DMSO) δ 1.4 (m, 2H), 2.50 (t, 2H, $J = 7.1$ Hz), 2.49 (m, 4H), 2.58 (t, 2H, $J = 7.1$ Hz), 4.02 (m, 1H), 7.77 (s, 4H), 7.78 (d, 1H, $J = 5.2$ Hz), 8.90 (bs, 2H), 9.14 (bs, 2H), 10.39 (s, 1H); MS (FAB) m/z 393.4 (MH^+).

Elemental Analysis

10 Required for

$\text{C}_{18}\text{H}_{24}\text{N}_4\text{O}_6 \cdot \text{F}_3\text{C}_2\text{O}_2\text{H} \cdot \text{H}_2\text{O}$: C 46.60 H 5.04 N 10.87

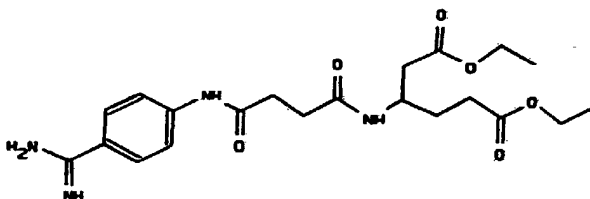
Found: C 46.64 H 5.11 N 10.77

Example 19

15

Diethyl-3-[[4-[[4-(aminoiminomethyl)phenyl]-amino]-1,4-dioxobutyl]amino]hexanedioate.

20



25

Step 1 Preparation of diethyl-3-aminoadipate.

Diethyl-3-oxoadipate (10 g, 46 mmol) was added to methanol (225 ml) followed by ammonium formate (27.4 g, 460 mmol) and NaBH_3CN (2.7 g, 46 mmol) at 25°C . After 24 h the methanol was removed in vacuo to leave a white mass. Methylene chloride was added and the mixture filtered. The methylene chloride was evaporated resulting in an oil which was dissolved in 1N HCl (200 ml), and was extracted with ether (100 ml). The ether layer was discarded, and the aqueous layer was made basic using solid K_2CO_3 . The product was extracted

into methylene chloride, dried over Na_2SO_4 , and evaporated to give diethyl-3-aminoadipate (7.5 g). ^1H NMR (d_6 -DMSO) δ 1.25 (t, 3H, $J = 8$ Hz), 1.26 (t, 3H, $J = 8$ Hz), 1.8 (m, 2H), 1.55 (bs, 2H), 3.18 (m, 1H), 4.13 (q, 4H, $J = 8$ Hz), 4.15 (q, 4H, $J = 8$ Hz); MS (FAB) m/z 218.3 (MH^+).

Step 2 Preparation of diethyl3-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]hexanedioate.

4-[[4-(Aminoiminomethyl)phenyl]-amino]-4-oxobutanoic acid hydrochloride prepared in Example 1, Step 1 (5.0 g, 18.5 mmol) was added to dry DMF (250 ml) followed by N-methylmorpholine (1.8 g, 18.5 mmol) and isobutyl chloroformate (2.7 g, 17 mmol) at 25°C . The mixture was stirred for 5 min. Diethyl-3-aminoadipate (4.0 g, 18.5 mmol) was added followed by dimethylaminopyridine. After 1 hour, the solvent was removed under reduced pressure and the product purified by reverse phase chromatography (.05% TFA water/acetonitrile) to result in 3.0 g of a white solid: ^1H NMR (d_6 -DMSO) δ 1.14 (t, 6H, $J = 7$ Hz), 1.55 (m, 1H), 1.7 (m, 1H), 2.3 (m, 2H), 2.57 (m, 2H), 2.67 (m, 2H), 4.02 (q, 4H, $J = 7$ Hz), 4.03 (m, 1H), 7.79 (s, 4H), 7.99 (d, 1H, $J = 8.1$ Hz), 9.06 (bs, 2H), 9.15 (bs, 2H), 10.22 (s, 1H); MS (FAB) m/z 435.2 (MH^+).

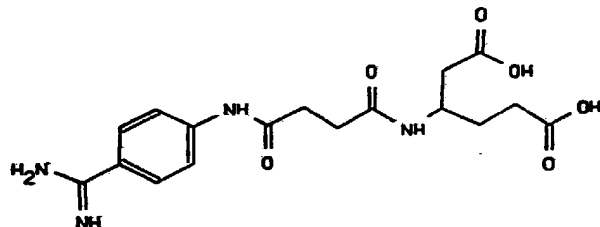
Elemental Analysis

Required for

$\text{C}_{21}\text{H}_{30}\text{N}_4\text{O}_6 \cdot \text{F}_3\text{C}_2\text{O}_2\text{H} \cdot \text{H}_2\text{O}$:	C 49.55	H 5.75	N 10.05
Found:	C 49.36	H 5.42	N 9.92

Example 20

3-[[4-[[4-(Aminoiminomethyl)phenyl]amino]-
1,4-dioxobutyl]amino]hexanedioic acid.



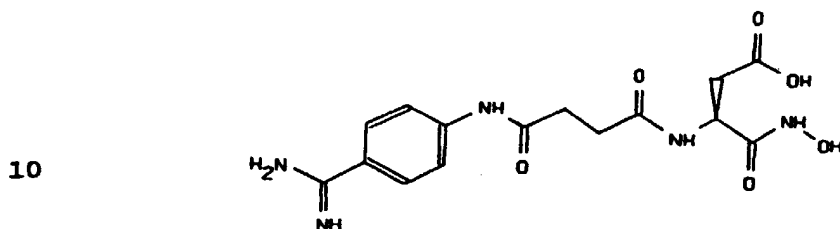
Diethyl 3-[[4-[[4-(aminoiminomethyl)phenyl]-
amino]-1,4-dioxobutyl]amino]hexanedioate (700 mg)
prepared in Example 19, Step 2 was added to
water/acetonitrile (20 ml) followed by lithium hydroxide
(150 mg) at 25°C. The mixture was stirred for 30 min.
The course of the reaction was monitored by RPHPLC.
After satisfactory product was formed the reaction was
neutralized with TFA and purified by reverse phase
chromatography (.05% TFA water/acetonitrile) to result
in 350 mg of a white solid: ¹H NMR (d₆-DMSO) δ 1.55 (m,
1H), 1.7 (m, 1H), 2.3 (m, 2H), 2.57 (m, 2H), 2.67 (m,
2H), 4.03 (m, 1H), 7.79 (s, 4H), 7.99 (d, 1H, J = 8 Hz),
8.86 (bs, 2H), 9.17 (bs, 2H), 10.2 (s, 1H); MS (FAB)
m/z 379.2 (MH⁺).

Elemental AnalysisRequired for

C ₁₇ H ₂₂ N ₄ O ₆ · F ₃ C ₂ O ₂ H · H ₂ O:	C 45.51	H 4.79	N 11.18
Found:	C 45.57	H 4.49	N 11.18

Example 21

3-(S)-[[4-[[4-(aminoiminomethyl)phenyl]amino]-
1,4-dioxobutyl]amino]-4-(hydroxyamino)-4-oxobutanoic
5 acid.



15 Step 1 Preparation of benzyl-3-amino-4-oxo-(N-hydroxylamino)-(3S)-butyrate.

N-t-Boc-L-aspartic acid, β -benzyl ester (10 g, 3.0 mmol) was dissolved in 100 ml of methylene chloride and added dropwise over a period of 10 min to a solution
20 of DCC (6.3 g, 3.0 mmol) in methylene chloride (20 ml) at 25°C under a N₂ atmosphere. After the solution was stirred for 0.5 h, trimethylsilyl hydroxylamine (3.0 g, 3 mmol) was added to the reaction mixture followed by DMAP (0.5 g). After the reaction was complete, ether
25 was added to the mixture, and the DCU was removed by filtration through a pad of celite. The solution was concentrated to give an oil which was dissolved in dioxane. To that solution was added 4N HCl in dioxane (20 ml). The product was isolated by filtration (4g) as
30 its hydrochloride salt. ¹H NMR (d₆-DMSO) δ 2.95 (m, 2H), 4.36 (m, 1H), 5.12 (d, 2H, J = 7 Hz), 7.37 (bs, 5H).

Step 2 3-(S)-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-4-(hydroxyamino)-4-oxobutanoic acid.

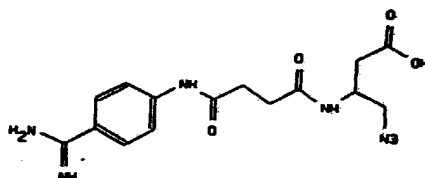
35 4-[[4-(aminoiminomethyl)phenyl]amino]-4-oxobutanoic acid hydrochloride prepared in Example 1, Step 1 (5.0 g, 18.5 mmol) was added to dry DMF (250 ml)

- followed by N-methylmorpholine (1.8 g, 18.5 mmol) and isobutyl chloroformate (2.7 g, 18.5 mmol) at 25°C. The mixture was stirred for 5 min. Benzyl-3S-amino-4-oxo-4-(N-hydroxylamino)butyrate hydrochloride (3.0 g, 18.5 mmol; from Step 1) was added followed by dimethylaminopyridine. After 1 h, the solvent was removed under reduced pressure and the product purified by reverse phase chromatography (.05% TFA water/acetonitrile) to result in 2.1 g of a white solid.
- 10 A portion of this material was then subjected to saponification conditions as previously described and purified by reverse phase chromatography (water/acetonitrile) to result in 225 mg of a white solid: ¹H NMR (d₆-DMSO) δ 2.44 (m, 3H), 2.60 (m, 2H), 2.4 (m, 1H), 4.49 (m, 1H), 6.98 (d, 1H, J = 8.0 Hz), 7.78 (s, 4H), 8.62 (d, 1H, J = 7.7 Hz), 8.94 (bs, 2H), 9.16 (bs, 2H), 10.20 (s, 1H); MS (FAB) m/z 348.1 (MH⁺). Elemental Analysis Required for
- 20 C₁₅ H₁₉N₅O₅ · F₃C₂O₂H · H₂O: C 44.25 H 3.90 N 15.18
Found: C 44.13 H 4.07 N 13.68

Example 22

- 25 3-(R)-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-4-azidobutanoic acid.

30



35

Step 1 Preparation of Benzyl-3-amino-4-azido butyrate.

The mesylate (27 g, 64 mmol) from Step 1, Example 16 was added to dry DMF followed by NaN_3 (12.6 g, 194 mmol), TDA-1 (0.5 g), DMAP (1.0 g), and heated at 70°C for 2-3 h. After complete reaction, water was added and the product was extracted with ether (2x150 ml). The ether extracts were washed with water, then dried over MgSO_4 , and the solvent evaporated to give benzyl-3-N-t-Boc-amino-4-azido-(3S)-butyrate (20g).

The crude Benzyl-3-N-t-Boc-amino-4-azido-(3S)-butyrate was dissolved in dioxane (100 ml) and to this solution 4N HCl in dioxane was added. After 6h, the dioxane was removed in vacuo to leave an oil to give Benzyl-3-amino-4-azido-(3S)-butyrate hydrochloride (11 g). ^1H NMR (d_6 -DMSO) δ 2.9 (dd, 2H, $J = 5.65$, 15 Hz), 3.83 (bs, 2H), 4.2 (m, 1H), 5.16 (s, 2H), 7.36 (bs, 5H); MS (FAB) m/z 235.1 (MH^+).

Step 2 Preparation of 3-(R)-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-4-azidobutanoic acid.

4-[[4-(aminoiminomethyl)phenyl]amino]-4-oxobutanoic acid hydrochloride prepared in Example 1, Step 1 (4.6 g, 18.5 mmol) was added to dry DMF (250 ml) followed by N-methylmorpholine (1.8 g, 18.5 mmol) and isobutyl chloroformate (2.8 g, 17 mmol) at 25°C. The mixture was stirred for 5 min. Benzyl-3-amino-4-azido-(3S)-butyrate hydrochloride (1 eq; from Step 1, Example 22) was added followed by dimethylaminopyridine. After 1 h the solvent was removed under reduced pressure and the product purified by reverse phase chromatography (water/acetonitrile) to result in 3.0 g of a white solid. A portion (750 mg) of the benzyl ester was subjected to the saponification conditions as previously described and purified by reverse phase chromatography (water/acetonitrile) to result in 340 mg of a white solid: ^1H NMR (d_6 -DMSO) δ 2.44 (m, 4H), 2.6 (m, 2H), 3.35 (bs, 2H), 4.18 (m, 1H), 7.78 (s, 4H), 8.1 (d, 1H, J

= 8 Hz), 8.27 (bs, 2H), 9.15 (bs, 2H), 10.40 (s, 1H);
MS (FAB) m/z 362.1 (MH⁺).

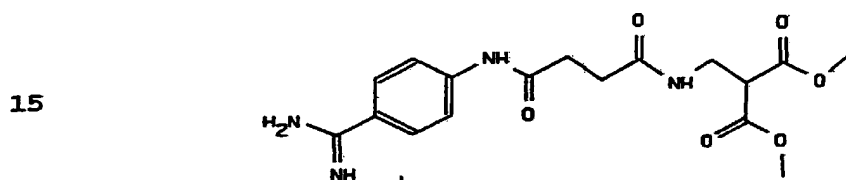
Elemental Analysis

Required for

5 C₁₅ H₁₉N₇O₄ · F₃C₂O₂H · H₂O: C 42.63 H 4.18 N 20.48
Found: C 42.49 H 4.06 N 20.06

Example 23

10 Dimethyl [[[4-[[4-(aminoiminomethyl)phenyl]-
amino]-1,4-dioxobutyl]amino]methyl]propanedioate.



20

Step 1 Preparation of Dimethyl-2-(aminomethyl)
malonate.

Dimethyl malonate (10 g, 75.8 mmol) was added
to DMF/water (70:30), K₂CO₃ (15 g, 100 mmol), PTC (1 g),
25 NaI (1 g), and tert-butyl bromoacetate (14.7 g, 75.8
mmol) at 25°C. The mixture was vigorously stirred for
24 h. After this time water (250 ml) was added and the
product was extracted with ether and dried over MgSO₄.
The oil which resulted after concentrating the solution
30 was dissolved in methylene chloride (80 ml) and TFA
(20 ml), and the resulting mixture was stirred for 3-6
hr. After this time, the solution was concentrated in
vacuo to give a viscous oil (8 g). A portion of this
oil (5 g, 26.3 mmol) was dissolved in DMF (50 ml).
35 Diphenyl phosphorylazide (7.5 g, 26.3 mmol) was added
and the resulting mixture was cooled to 0°C.
Triethylamine (2.7 g, 26.3 mmol) was added and the

solution stirred for 2 h. After this time, water was added (200 ml) and the acyl azide extracted into ether, dried over MgSO_4 , and the solvent removed. The oil was dissolved in dioxane followed by the addition of 4N HCl in dioxane (10 ml). The product, a white solid, soon separated out as its hydrochloride salt (3 g). ^1H NMR (d_6 -DMSO) δ 3.25 (m, 2H), 3.70 (s, 3H), 4.0 (t, 1H, $J = 7$ Hz), 8.4 (bs, 2H); MS (FAB) m/z 162.1 (MH^+), 145.2, 133.3.

- 10 Step 2 Preparation of Dimethyl [[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]propanedioate.

4-[[4-(Aminoiminomethyl)phenyl]amino]-4-oxobutanoic acid hydrochloride prepared in Example 1, Step 1 (5.0 g, 18.5 mmol) was added to dry DMF (250 ml) followed by N-methylmorpholine (1.7 g, 18.5 mmol) and isobutyl chloroformate (2.8 g, 17 mmol) at 25°C . The mixture was stirred for 5 min. Dimethyl2-(aminomethyl)malonate (from Step 1; 1 eq) was added followed by dimethylaminopyridine. After 1 h, the solvent was removed under reduced pressure and the product purified by reverse phase chromatography (water/acetonitrile) to result in 2.0 g of a white solid: ^1H NMR (d_6 -DMSO) δ 2.57 (m, 2H), 2.67 (m, 2H), 3.47 (m, 2H), 3.5 (s, 6H), 3.51 (m, 1H), 7.79 (s, 4H), 8.1 (t, 1H, $J = 7$ Hz), 8.7 (bs, 2H), 9.09 (bs, 2H), 10.32 (s, 1H); MS (FAB) m/z 379.0 (MH^+).

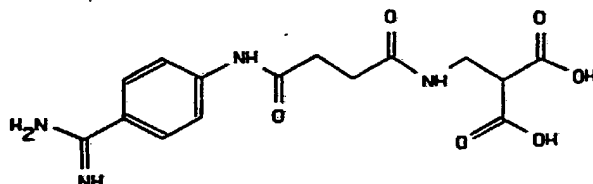
Elemental Analysis

Required for

- 30 $\text{C}_{17}\text{H}_{22}\text{N}_4\text{O}_6 \cdot \text{F}_3\text{C}_2\text{O}_2\text{H} \cdot \text{H}_2\text{O}$: C 45.50 H 4.72 N 11.18
Found: C 45.20 H 4.66 N 11.17

Example 24

[[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]methyl]propanedioic acid.



Dimethyl [[4-[[4-(aminoiminomethyl)phenyl]-amino]-1,4-dioxobutyl]aminomethyl]propanedioate prepared in Example 23, Step 2 (500 mg) was added to water/acetonitrile (20 ml) followed by lithium hydroxide (100 mg) at 25°C. The mixture was stirred for 30 min. The course of the reaction was monitored by RPHPLC. After satisfactory product was formed the reaction was neutralized with TFA and purified by reverse phase chromatography (0.5% TFA water/acetonitrile) to result in 350 mg of a white solid: ¹H NMR (d₆-DMSO) δ 2.41 (m, 2H), 2.57 (m, 2H), 3.35 (m, 1H), 3.47 (m, 2H), 7.79 (s, 4H), 8.1 (t, 1H, J = 6.1 Hz), 7.78 (s, 4H), 8.02 (m, 1H), 8.87 (bs, 2H), 9.15 (bs, 2H), 10.39 (s, 1H); MS (FAB) m/z 350.0 (MH⁺).

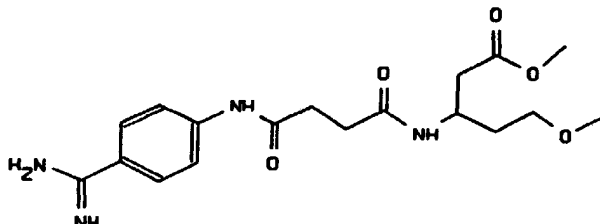
Elemental Analysis

Required for

30	C ₁₅ H ₁₈ N ₄ O ₆ · F ₃ C ₂ O ₂ H · H ₂ O:	C 44.32	H 4.32	N 13.66
	Found:	C 44.72	H 4.72	N 13.06

Example 25

Methyl 3-[[4-[[4-(aminoiminomethyl)phenyl]-amino]-1,4-dioxobutyl]amino]-4-methoxypentanoate.



Step 1 Preparation of methyl-5-methoxy-3-aminovalerate.

Methyl-5-methoxy-3-oxovalerate (10 g, 62.5 mmol) was added to methanol (200 ml) followed by ammonium formate (39.4 g, 620 mmol) and NaBH_3CN (3.9 g, 46 mmol) at 25°C. After 24 h the methanol was removed in vacuo to leave a white mass. Methylene chloride was added and the mixture filtered. The methylene chloride was evaporated resulting in an oil which was dissolved in 1N HCl (200 ml) and extracted with ether (100 ml). The ether layer was discarded and the aqueous layer was made basic using solid K_2CO_3 . The product was extracted into methylene chloride dried over Na_2SO_4 and evaporated to give methyl-5-methoxy-3-aminovalerate (7.5 g). ^1H NMR (d6-DMSO) δ 1.25 (m, 3H) 1.66 (m, 2H), 1.86 (bs, 2H), 2.9 (M, 2H), 3.33 (s, 3H), 3.49 (M, 2H), 3.7 (s, 3H); MS (FAB) m/z 161.2 (MH^+).

Step 2 Preparation of methyl 3-[[4-[[4-(aminoiminomethyl)phenyl]-amino]-1,4-dioxobutyl]amino]-4-methoxypentanoate.

4-[[4-(Aminoiminomethyl)phenyl]amino]-4-oxobutanoic acid hydrochloride prepared in Example 1, Step 1 (5.0 g, 18.5 mmol) was added to dry DMF (250 ml) followed by N-methylmorpholine (1.7 g, 18.5 mmol) and

isobutyl chloroformate (2.8 g, 17 mmol) at 25°C. The mixture was stirred for 5 min. Methyl-5-methoxy-3-aminovalerate (3.0 g, 18.5 mmol) was added followed by dimethylaminopyridine. After 1 h, the solvent was removed under reduced pressure and the product purified by reverse phase chromatography (.05% TFA water/acetonitrile) to result in 2.8 g of a white solid:

¹H NMR (d₆-DMSO) δ 1.74 (m, 2H), 2.4 (m, 4H), 2.6 (m, 2H), 3.16 (s, 3H), 3.33 (m, 2H), 3.56 (s, 3H), 4.12 (m, 1H), 7.78 (s, 4H), 7.81 (d, 1H, J=8.5 Hz), 9.02 (bs, 2H), 9.15 (bs, 2H), 10.40 (s, 1H); MS (FAB) m/z 379.5 (MH⁺).

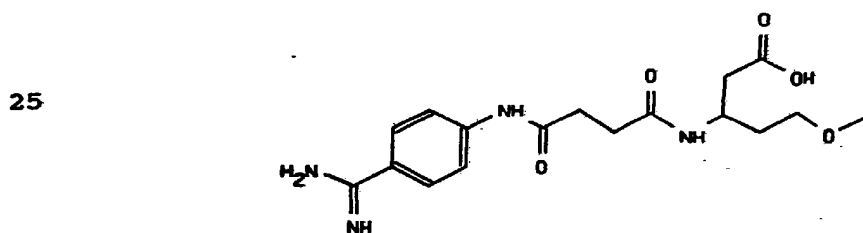
Elemental Analysis

Required for

15 C₁₈ H₂₆N₄O₅ · F₃C₂O₂H · H₂O: C 48.78 H 5.49 N 11.38
 Found: C 48.35 H 5.55 N 10.32

Example 26

20 3-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-4-methoxypentanoic acid.



30 Methyl-3-[[4-[[4-(aminoiminomethyl)phenyl]-amino]-1,4-dioxobutyl]amino]-4-methoxypentanoate prepared in Example 25, Step 2 (500 mg) was added to water/acetonitrile (20 ml) followed by lithium hydroxide (100 mg) at 25°C. The mixture was stirred for 30 min. The course of the reaction was monitored by RPHPLC. After satisfactory product was formed the reaction was

neutralized with TFA and purified by reverse phase chromatography (water/acetonitrile) to result in 450 mg of a white solid: ^1H NMR (d_6 -DMSO) δ 1.74 (m, 2H), 2.4 (m, 4H), 2.6 (m, 2H), 3.16 (s, 3H), 3.33 (m, 2H), 4.12 (m, 1H), 7.78 (s, 4H), 7.81 (d, 1H, $J = 8.5$ Hz), 9.02 (bs, 2H), 9.15 (bs, 2H), 10.40 (s, 1H); MS (FAB) m/z 365.4 (MH^+).

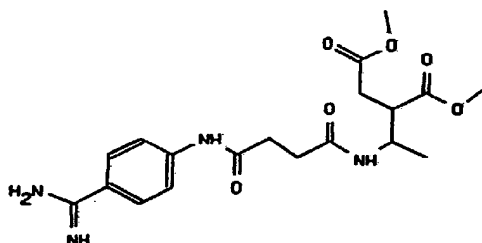
Elemental Analysis

Required for

10 $\text{C}_{17}\text{H}_{24}\text{N}_4\text{O}_5 \cdot \text{F}_3\text{C}_2\text{O}_2\text{H} \cdot \text{H}_2\text{O}$: C 46.82 H 5.33 N 11.49
Found: C 46.68 H 5.13 N 11.05

Example 27

Dimethyl 2-[1-[[4-[[4-(aminoiminomethyl)phenyl]-amino]-1,4-dioxobutyl]amino]-ethyl]-succinate.



Step 1 Preparation of dimethyl-2-(aminoethyl) succinate.

Dimethyl, acetylsuccinate (10 g, 53 mmol) was added to methanol (200 ml) followed by ammonium formate (34 g, 530 mmol) and NaBH_3CN (3.4 g, 53 mmol) at 25°C. After 24 h the methanol was removed in vacuo to leave a white mass. Methylene chloride was added and the mixture filtered. The methylene chloride was evaporated resulting in an oil which was dissolved in 1N HCl (200 ml) and extracted with ether (100 ml). The ether layer was discarded and the aqueous layer was made basic using solid K_2CO_3 . The product was extracted into methylene chloride, dried over Na_2SO_4 , and evaporated to give dimethyl-2-(aminoethyl)succinate (6 g). ^1H NMR (d_6 -DMSO) δ 0.97 (d, 3H, $J = 7.1$ Hz), 2.66 (m, 2H), 3.05 (m, 1H), 3.56 (s, 3H), 3.60 (s, 3H), 4.15 (m, 1H); MS (FAB) m/z 190.2 (MH^+), 158.2, 141.2.

Step 2 Preparation of dimethyl-(2-[1-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-ethyl]-succinate.

4-[[4-(aminoiminomethyl)phenyl]-amino]-4-oxobutanoic acid hydrochloride prepared in Example 1, Step 1 (5.0 g, 18.5 mmol) was added to dry DMF (250 ml)

followed by N-methylmorpholine (1.8 g, 18.5 mmol) and isobutyl chloroformate (2.7 g, 18.5 mmol) at 25°C. The mixture was stirred for 5 minutes. Dimethyl(2-aminoethyl)succinate (3.5 g, 18.5 mmol) was added followed by dimethylaminopyridine. After 1 h, the solvent was removed under reduced pressure and the product purified by reverse phase chromatography (.05% TFA water/acetonitrile) to result in 2.1 g of a white solid. ¹H NMR (d₆-DMSO) δ 0.98 (d, 3H, J = 7.1 Hz), 2.44 (m, 2H), 2.55 (m, 4H), 3.1 (m, 1H), 3.55 (s, 3H), 3.58 (s, 3H), 4.15 (m, 1H), 7.78 (s, 4H), 7.92 (d, 1H, J = 7.7 Hz), 8.84 (bs, 2H), 9.16 (bs, 2H), 10.20 (s, 1H); MS (FAB) m/z 407.2 (MH⁺).

Elemental Analysis

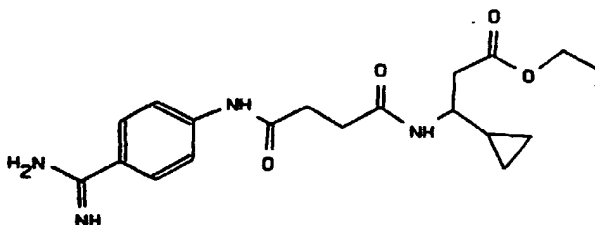
Required for

C₁₉H₂₆N₄O₆·F₃C₂O₂H·H₂O: C 48.46 H 5.19 N 10.76

Found: C 48.83 H 5.33 N 10.75

Example 28

Ethyl β-[[4-[[4-(aminoimino-methyl)phenyl]amino]-1,4-dioxobutyl]amino]cyclopropanepropanoate.

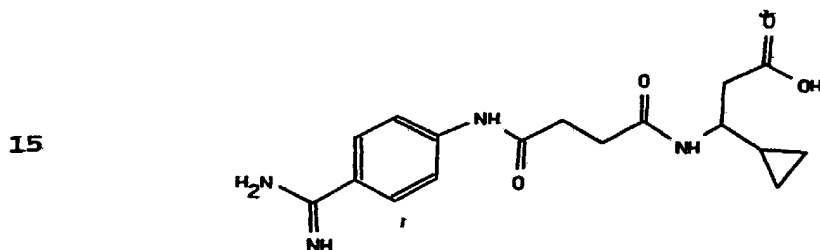


The title compound was prepared in the manner of Example 1 substituting ethyl 3-amino-3-cyclopropylpropanoate for D,L-3-amino-3-phenylpropionic acid. The product was purified by reverse phase

HPLC using the conditions of Example 1 to afford the title compound. The product was verified by ^{13}C NMR (CD_3OD) δ 2.5, 3.2, 13.6, 15.0, 30.8, 32.5, 40.1, 51.4, 60.8, 119.8, 122.7, 129.2, 144.9, 166.8, 172.1, 172.6, 173.0, Chemical ionization mass spectrometry (MH^+)=375.

Example 29

β -[[4-[[4-(Aminoiminomethyl)phenyl]amino]-1, 4-dioxobutyl]amino]cyclopropanepropanoic acid.



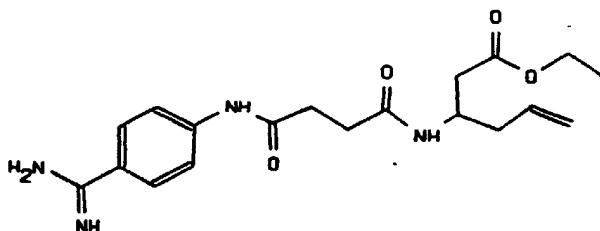
20 Porcine liver esterase (200 μL , Sigma, 11 mg/mL in 3.2 M $(\text{NH}_4)_2\text{SO}_4$ at pH=8) was added to the final product of Example 28 in 20 mL of 0.1M phosphate buffer (pH=7.4). After 20h at 23°C, the reaction mixture was concentrated in vacuo. The residue was dissolved in 1N HCl (3mL) and subsequently diluted with acetonitrile (5mL) followed by immediate purification by reverse phase HPLC using the conditions of Example 1 to afford 23.0mg (83%) of the title compound. The product was verified by ^{13}C NMR (CD_3OD) δ 1.8, 2.6, 15.0, 30.3, 31.8, 39.1, 50.6, 119.2, 122.1, 128.6, 144.3, 166.8, 172.0, 172.4, 173.4; Fast Atom Bombardment Mass Spectrometry (MH^+)=347.

Example 30

Ethyl 3-[[4-[[4-(aminoimino-
methyl)phenyl]amino]-1,4-dioxobutyl]amino]-5-hexenoate.

5

10



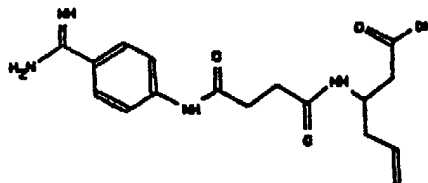
The title compound was prepared in the manner
15 of Example 1 substituting ethyl 3-amino-5-hexenoate for
D,L-3-amino-3-phenylpropionic acid. The product was
purified by reverse phase HPLC using the conditions of
Example 1 to afford the title compound. The product was
verified by C NMR (CD₃OD) δ 12.6, 29.8, 31.3, 37.9,
20 45.9, 59.9, 116.5, 118.7, 121.5, 128.1, 133.4, 143.7,
165.6, 171.1, 171.6, 172.2; Fast Atom Bombardment Mass
Spectrometry (MH⁺) = 375.

Example 31

25

3-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,
4-dioxobutyl]amino]-5-hexenoic acid.

30



35

The title compound was prepared by treating the final product of the previous example with porcine liver esterase in the manner of Example 29. The product was purified by reverse phase HPLC using the conditions of Example 1 to afford the title compound. The product was verified by ^{13}C NMR (CD_3OD) δ 31.3, 32.8, 39.0, 39.2, 47.0, 117.8, 120.2, 123.1, 129.5, 135.1, 145.2, 165.6, 173.0, 173.6, 174.4; Fast Atom Bombardment Mass Spectrometry (MH^+)=347.

10 Elemental Analysis

Required for

$\text{C}_{17}\text{H}_{22}\text{N}_4\text{O}_4 \cdot 1.0 \text{ CF}_3\text{CO}_2\text{H} \cdot 0.6\text{H}_2\text{O}$: C 48.43 H 5.18 N 11.89

Found:

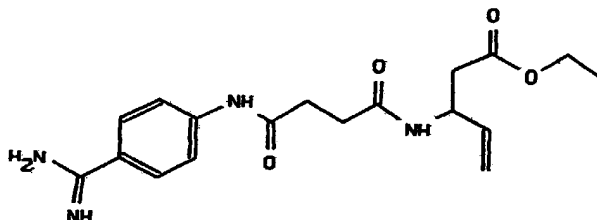
C 48.14 H 4.86 N 11.72

15

Example 32

Ethyl 3-[[4-[[4-(aminoimino-methyl)phenyl]amino]-1,4-dioxobutyl]amino]-4-pentenoate.

20



25

The title compound was prepared in the manner of Example 1 substituting ethyl 3-amino-4-pentenoate for D,L-3-amino-3-phenylpropionic acid. The product was purified by reverse phase HPLC using the conditions of Example 1 to afford the title compound. The product was verified by ^{13}C NMR (CD_3OD): δ 12.8, 29.5, 31.0, 47.5, 59.5, 69.3, 113.8, 118.2, 121.1, 128.2, 136.5, 143.7, 165.9, 169.9, 170.9, 171.0; Fast Atom Bombardment Mass Spectrometry (MH^+)=361.

Anal. Calcd. for

$C_{18}H_{24}N_4O_4$. 1.0 CF_3CO_2H . 0.5 H_2O : C 49.69 H 5.42 N 11.59

Found:

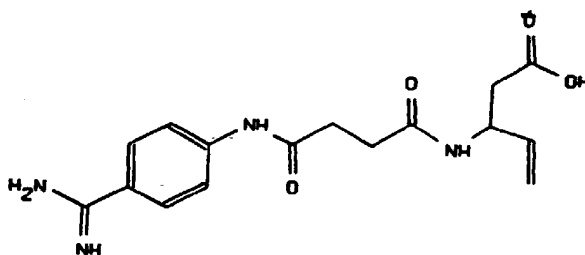
C 49.93 H 5.30 N 11.39

5

Example 33

3-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-4-pentenoic acid.

10



15

The title compound was prepared by treating the final product of the previous example with porcine liver esterase in the manner of Example 29. The product was purified by reverse phase HPLC using the conditions of Example 1 to afford the title compound. The product was verified by ^{13}C NMR (CD_3OD) δ 30.0, 31.3, 38.2, 47.9, 114.0, 118.9, 119.0, 128.1, 136.3, 165.9, 171.8, 172.1, 173.2; Fast Atom Bombardment Mass Spectrometry (MH^+)=333.

Anal. Calcd. for

$C_{16}H_{20}N_4O_4$. 1.0 CF_3CO_2H . 1.45 H_2O : C 45.76 H 5.10 N 11.86

Found:

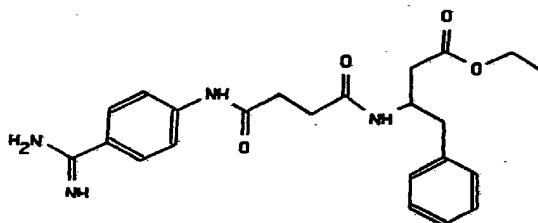
C 45.37 H 4.74 N 12.29

30

Example 34

Ethyl β -[[4-[[4-(aminoimino-
methyl)phenyl]amino]-1, 4-dioxobutyl]amino]benzene-
5 butanoate.

10



15

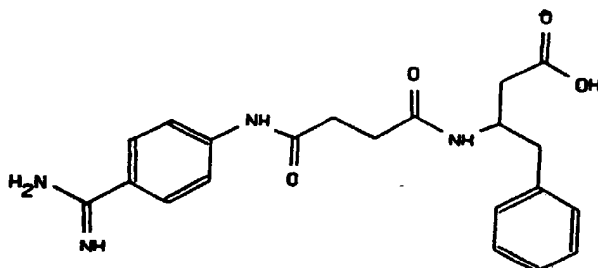
The title compound was prepared in the manner
of Example 1 substituting ethyl 3-amino-4-
phenylbutanoate for D,L-3-amino-3-phenylpropionic acid.
The product was purified by reverse phase HPLC using the
conditions of Example 1 to afford the title compound.

20 The product was verified by ^{13}C NMR (CD_3OD) δ 13.6, 30.8,
32.2, 38.8, 40.4, 48.7, 60.8 119.7, 122.7, 126.6, 128.5,
129.0, 129.5, 138.4, 144.8, 166.1, 171.2, 172.0, 172.5;
Fast Atom Bombardment Mass Spectrometry (MH^+)=425.
Anal. Calcd. for

25 $\text{C}_{23}\text{H}_{28}\text{N}_4\text{O}_4 \cdot 1.0 \text{ CF}_3\text{CO}_2\text{H} \cdot 0.5 \text{ H}_2\text{O}$: C 54.84 H 5.52 N 10.23
Found: C 54.78 H 5.40 N 10.20

Example 35

β -[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]benzenebutanoic acid.



The title compound was prepared by treating the final product of the previous example with porcine liver esterase in the manner of Example 29. The product was purified by reverse phase HPLC using the conditions of Example 1 to afford the title compound. The product was verified by ^{13}C NMR (CD_3OD) δ 30.2, 31.7, 37.8, 39.6, 47.1, 119.2, 122.1, 126.1, 128.6, 129.0, 138.0, 144.3, 166.2, 172.0, 172.5, 173.2; Fast Atom Bombardment Mass Spectrometry (MH^+)=397.

Anal. Calcd. for

$\text{C}_{21}\text{H}_{24}\text{N}_4\text{O}_6 \cdot 1.4 \text{ CF}_3\text{CO}_2\text{H}$: C 51.41 H 4.60 N 10.08

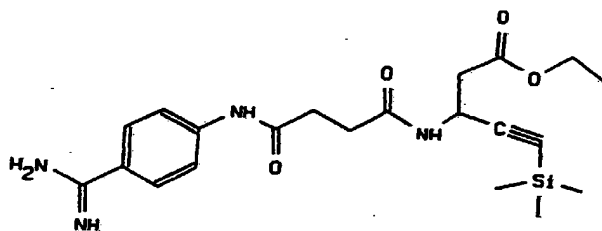
Found:

C 51.75 H 4.64 N 10.43

Example 36

Ethyl 3-[[4-[[4-(aminoimino-
methyl)phenyl]amino]-1, 4-dioxobutyl]amino]-5-
5 (trimethylsilyl)-4-pentynoate.

10



15

The title compound was prepared in the manner of Example 1 substituting ethyl 5-(trimethylsilyl)-4-pentynoate for D,L-3-amino-3-phenylpropionic acid. The product was purified by reverse phase HPLC using the conditions of Example 1 to afford the title compound.

20 The product was verified by ^{13}C NMR (CD_3OD) δ -1.5, 13.2, 30.0, 31.4, 38.6, 40.1, 60.6, 87.0, 103.3, 119.2, 122.0, 128.6, 144.2, 166.1, 170.0, 172.0, 172.1; Fast Atom Bombardment Mass Spectrometry (MH^+)=431.

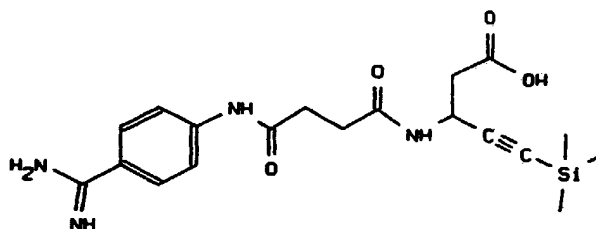
Anal. Calcd. for

25 $\text{C}_{21}\text{H}_{30}\text{N}_4\text{O}_4\text{Si} \cdot 1.3 \text{ CF}_3\text{CO}_2\text{H}$: C 48.97 H 5.45 N 9.68
Found: C 48.68 H 5.41 N 9.71

Example 37

3-[[4-[4-(aminoiminomethyl)phenyl]amino]-
1,4-dioxobutyl]aminol]-5-(trimethylsilyl)-4-pentynoic
5 acid.

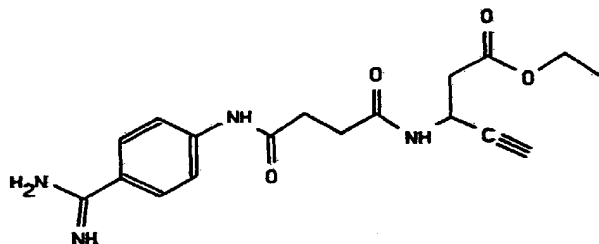
10



15 The title compound was prepared by treating
the final product of the previous example with porcine
liver esterase in the manner of Example 29. The product
was purified by reverse phase HPLC using the conditions
of Example 1 to afford the title compound. The product
20 was verified by ^{13}C NMR (CD_3OD) δ -0.6, 30.8, 32.3, 39.4,
40.7, 87.6, 104.5, 120.1, 123.0, 129.5, 145.2, 166.2,
172.8, 172.9, 173.0; Fast Atom Bombardment Mass
Spectrometry (MH^+)=403
Anal. Calcd. for
25 $\text{C}_{19}\text{H}_{26}\text{N}_4\text{O}_4\text{Si} \cdot 1.4 \text{ CF}_3\text{CO}_2\text{H}$: C 45.66 H 4.82 N 9.77
Found: C 45.84 H 4.92 N 9.86

Example 38

Ethyl 3-[[4-[[4-(aminoimino-methyl)phenyl]amino]-1, 4-dioxobutyl]amino]-4-pentynoate.



The title compound was prepared in the manner of Example 1 substituting ethyl 3-amino-4-pentynoate for D,L-3-amino-3-phenylpropionic acid. The product was purified by reverse phase HPLC using the conditions of Example 1 to afford the title compound. The product was verified by ^{13}C NMR (CD_3OD) δ 13.6, 30.3, 31.9, 38.1, 40.4, 61.0, 71.9, 82.0, 119.6, 122.5, 129.1, 144.8, 166.5, 170.3, 172.1, 172.2; Fast Atom Bombardment Mass Spectrometry (MH^+)=359.

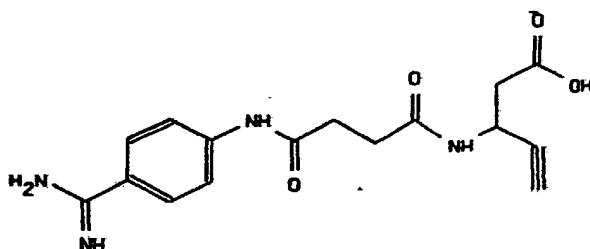
Anal. Calcd. for

$\text{C}_{16}\text{H}_{18}\text{N}_4\text{O}_4 \cdot 1.5 \text{ CF}_3\text{CO}_2\text{H} \cdot 0.65 \text{ H}_2\text{O}$: C 44.48 H 4.09 N 10.92

Found: C 44.05 H 4.19 N 11.38

Example 39

(\pm)-3-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1, 4-dioxobutyl]amino]-4-pentynoic acid.



The title compound was prepared by treating the final product of the previous example with porcine liver esterase in the manner of Example 29. The product was purified by reverse phase HPLC using the conditions of Example 1 to afford the title compound. The product was verified by ^{13}C NMR (CD_3OD) δ 29.9, 31.4, 37.7, 39.5, 71.1, 81.5, 119.2, 122.1, 128.3, 144.2, 166.2, 171.8, 172.0, 172.1; Fast Atom Bombardment Mass Spectrometry (MH^+)=331.

Anal. Calcd. for

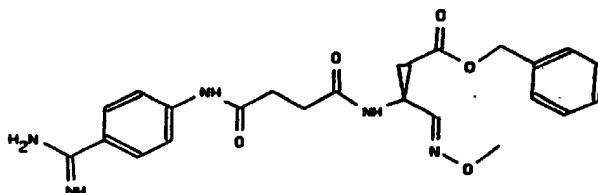
$\text{C}_{16}\text{H}_{18}\text{N}_4\text{O}_4 \cdot 1.5 \text{ CF}_3\text{CO}_2\text{H} \cdot 0.65 \text{ H}_2\text{O}$: C 44.48 H 4.09 N 10.92

Found:

C 44.05 H 4.19 N 11.38

Example 40

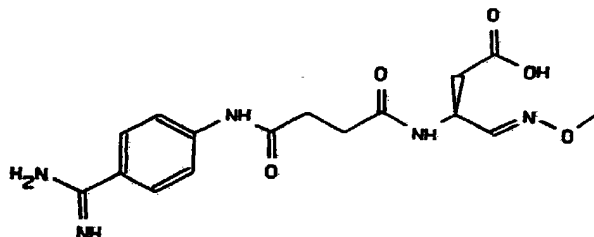
Phenylmethyl 3S-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1, 4-dioxobutyl]aminol-4-(methoxyimino)butanoate.



The title compound was prepared in the manner of Example 1 substituting phenylmethyl 3(S)-amino-4-(methoxyimino)butanoate for 3-amino-3-phenylpropionic acid. The product was purified by reverse phase HPLC using the conditions of Example 1 to afford the title compound. The product was verified by ^{13}C NMR (CD_3OD) δ 30.0, 31.5, 36.3, 43.5, 46.2, 61.2, 61.7, 66.7, 119.2, 122.1, 127.8, 128.1, 128.6, 136.0, 144.3, 148.0, 150.2, 166.1, 170.5, 172.0, 172.8; Fast Atom Bombardment Mass Spectrometry (MH^+)= 454.

Example 41

3S-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-4-(methoxyimino)butanoic acid.



The title compound was prepared by treating the final product of Example 40 with porcine liver esterase in the manner of Example 29. The product was purified by reverse phase HPLC using the conditions of example 1 to afford the title compound. The product was verified by C NMR (CD₃OD) δ 30.2, 31.8, 36.2, 47.4, 61.0, 119.2, 122.4, 128.8, 144.6, 148.8, 166.0, 171.8, 172.1, 172.4; Fast Atom Bombardment Mass Spectrometry (MH⁺)=364.

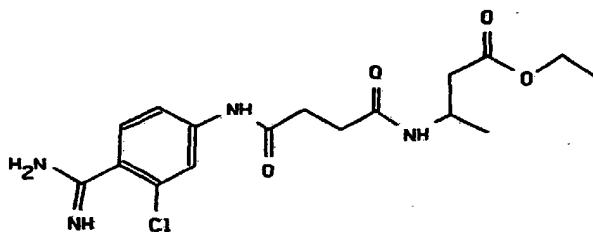
Anal. Calcd. for

C₁₆H₂₁N₅O₅ · 1.25 CF₃CO₂H · 0.5 H₂O: C 43.15 H 4.55 N 13.60

Found: C 43.26 H 4.19 N 13.36

Example 42

Ethyl 3-[[4-[[4-(aminoiminomethyl)-3-chlorophenyl]amino]-1,4-dioxobutyl]amino]butanoate.



Step 1 Preparation of 4-[[4-(aminoiminomethyl)-3-chlorophenyl]amino]-4-oxobutanoic acid hydrochloride.

To a mixture of 4-amino-2-chlorobenzonitrile (3.05g, 20.0 mmol), diisopropylethylamine (5.0mL, 30.0 mmol), and methylene chloride (20mL) at 0°C under nitrogen was added 3-carbomethoxypropionyl chloride (4.50g, 30.0 mmol) dropwise over 3 min. After 10 min at 0°C, the reaction mixture was allowed to warm up to 23°C. After 2h, the reaction mixture was concentrated in vacuo, diluted with ethyl acetate (150mL), washed with 1N KHSO₄ (1 x 80 mL), brine (1x80mL), dried (Na₂SO₄), and concentrated in vacuo. The residue was diluted with ether (80mL), filtered, and concentrated in vacuo. This procedure afforded material (4.5g, 85%) of sufficient purity to be taken on to the next step without further purification.

The ester (4.0g, 15 mmol) was hydrolyzed by treating with 1N NaOH: methanol (15mL:60mL) for 45 min. The reaction was concentrated in vacuo, diluted with 5% NaHCO₃ (150mL), and extracted with ethyl acetate (60mL). The aqueous layer was acidified (pH1-2) with 1N KHSO₄ and extracted with ethyl acetate (2x100mL). After drying (MgSO₄), the ethyl acetate was removed in vacuo to afford the free acid (3.1g, 82%).

Hydrogen sulfide was bubbled through a solution of 4.0g (15.9 mmol) of the above acid in pyridine:triethylamine (24mL:2.4mL) for 5 minutes at 23°C. After 24 h at 23°C in an enclosed flask, the reaction mixture was concentrated under a steady stream of nitrogen. The residue was diluted with ethyl acetate (300mL), washed with KHSO₄ (1N, 2x 100mL), brine (1x50mL), and dried (Na₂SO₄). Concentration in vacuo afforded a quantitative mass recovery of the thioamide.

The thioamide (4.53g, 15.8mmol) was dissolved in a solution of acetone:iodomethane (28mL:2mL). The reaction mixture was warmed to achieve reflux for 40 minutes. Concentration in vacuo followed by trituration

with ether and filtration afforded a quantitative yield of the HI salt.

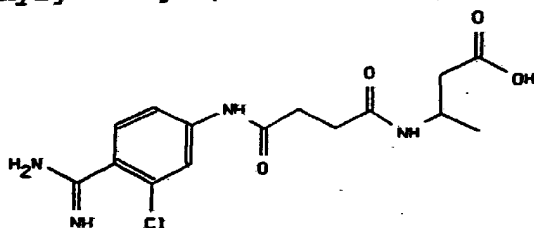
A solution of thioimide (15.8mmol) and ammonium acetate (1.83g, 23.7mmol) in methanol (10mL) was warmed to achieve reflux for 4h. After cooling to 23°C, the reaction mixture was concentrated under a steady stream of nitrogen in the hood. The residue was dissolved in H₂O (20 mL) and diluted with acetone (80mL) to afford the zwitter-ion (2.53g, 59.4%). The hydrochloride salt was formed by treatment with 6N HCl in dioxane (40mL) for 1h at 23°C. Concentration in vacuo afforded the hydrochloride salt which was azeotroped with benzene prior to use.

Step 2 Ethyl 3-[[4-[[4-(aminoiminomethyl)-3-chlorophenyl]amino]-1, 4-dioxobutyl]amino]butanoate.

The title compound was prepared in the manner of Example 1 substituting ethyl 3-aminobutanoate for D,L-3-amino-3-phenylpropionic acid and using the acid prepared in Step 1 of Example 42. The product was purified by reverse phase HPLC using the conditions of Example 1 to afford the title compound. The product was verified by ¹³C NMR (CD₃OD) δ 15.5, 21.5, 31.3, 33.0, 43.2, 61.2, 118.5, 120.5, 124.7, 132.0, 132.3, 144.6, 144.8, 165.7, 171.6, 172.9; Chemical Ionization Mass Spectrometry (MH⁺)=383.

Example 43

3-[[4-[[4-(aminoiminomethyl)-3-chlorophenyl]amino]-1,4-dioxobutyl]amino]butanoic acid.

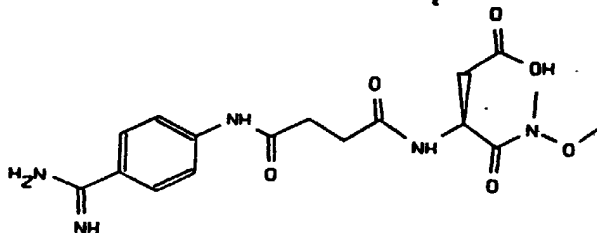


The title compound was prepared by treating the final product of the previous example with porcine liver esterase in the manner of Example 29. The product was purified by reverse phase HPLC using the conditions of example 1 to afford the title compound. The product was verified by ^{13}C NMR (CD_3OD) δ 18.6, 29.8, 31.3, 39.8, 42.0, 117.2, 119.9, 123.5, 129.7, 131.4, 143.5, 166.0, 172.0, 172.1, 173.1; Fast Atom Bombardment Mass Spectrometry (MH^+)=355.

Anal. Calcd. for $\text{C}_{15}\text{H}_{19}\text{N}_4\text{O}_4\text{Cl}$. 1.0 $\text{CF}_3\text{CO}_2\text{H}$. 1.0 H_2O : C 41.94 H 4.55 N 11.51
Found: C 41.63 H 4.23 N 11.89

Example 44

3S - [[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-4-(methoxymethylamino)-4-oxobutanoic acid.



Step 1 Preparation of 3S-amino-4-(methoxymethylamino)4-oxobutanoic acid.

To N-tBoc-L-aspartic acid, beta-benzyl ester (10 g, 31 mmole) dissolved in 50 mL methylene chloride was added triethylamine (4.31 mL, 31 mmol). To this was added benzotriazol-1-yloxytris(dimethylamino)-phosphonium hexafluorophosphate (BOP) (10.8 g, 31 mmol). After several minutes O,N-dimethylhydroxyl amine hydrochloride (3.40 g, 33.5 mmol) and triethylamine (4.31 mL, 32.3 mmole) was added and the reaction stirred

at 25°C for several hours. The reaction mixture was diluted to 200 mL by addition of methylene chloride and the reaction mixture was washed successively with dilute aqueous hydrochloric acid, saturated aqueous sodium hydrogen carbonate, and saturated aqueous sodium chloride solution. The organic layer was dried with magnesium sulfate and concentrated in vacuo to give the crude product. This product was dissolved in ethyl acetate and passed over a 4 x 4 cm pad of Merck 60 silica gel. The ethyl acetate was evaporated to give 8.9 g of desired product (78%).

The N-BOC amido benzyl ester prepared above (7.9 g, 21.6 mmol) was dissolved in 50 mL methanol. The solution was transferred along with 0.5 gm 3% palladium on carbon catalyst to a medium pressure hydrogenation apparatus equipped with a magnetic stirring bar, pressure gauge, and gas inlet and outlet valves. Hydrogen was introduced (54 psig) and the reaction allowed to continue overnight. The catalyst was removed by filtration over a celite pad and the solvent removed in vacuo to give the desired N-BOC amido acid: ¹H NMR (300 MHz, d₆DMSO) δ 1.45 (s, 9H), 2.8 (m, 2H), 3.20 (s, 3H), 3.72 (s, 3H), 4.55 (m, 1H). FABMS 283 (M+ Li).

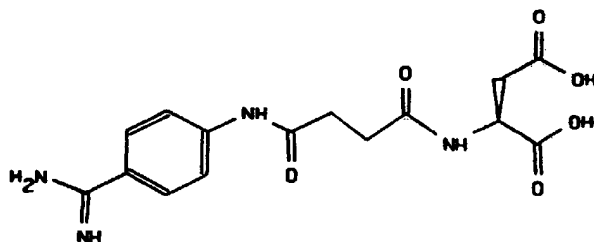
The N-BOC amido acid prepared above was dissolved in a minimum of 1,4-dioxane and 50 mL of 4N HCl in dioxane added at room temperature. The reaction was allowed to proceed until no further gas evolution was noted (30 minutes) and the solvent evaporated. The desired amino acid as the trifluoroacetate salt was isolated by preparative RPHPLC and lyophilized to give a white powder (2.33 grams, 8 mmol, 38 % overall isolated yield): ¹H NMR (300 MHz, d₆DMSO) δ 3.02 (bs, 2H), 3.12 (s, 3H), 3.69 (s, 3H), 4.18 (m, 1H). FABMS 177.1 (MH+).

Step 2 Preparation of 3S-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-4-(methoxymethyl-amino)4-oxobutanoic acid.

Coupling of 3S-amino-4-(methoxymethylamino)4-oxobutanoic acid to 4-[[4-(aminoiminomethyl)phenyl]-amino]-4-oxobutanoic acid was achieved in a fashion similar to Example 1 Step 2. Thus, 4-[[4-(aminoiminomethyl)phenyl]-amino]-4-oxobutanoic acid hydrochloride (0.70 g, 2.6 mmol) was reacted with isobutylchloroformate (0.34 mL, 2.6 mmol) and an equivalent of N-methylmorpholine (0.29 mL, 2.6 mmole) in DMF. Following activation 3S-amino-4-(methoxymethylamino)4-oxobutanoic acid (0.5 g, 1.7 mmol) was added with an equivalent of N-methylmorpholine and the reaction allowed to proceed overnight. The solvent was removed and the product isolated by preparative hplc and the fractions containing desired product taken to pH 6 with lithium hydroxide. The lithium salt was isolated by lyophilization. ¹H NMR (300 MHz, d₆ DMSO) δ 2.65 (m, 6H), 3.05, 3.10 (s', 3H), 3.65, 3.7 (s, 3H), 7.8 (m, 5H), 9.5 (m, 4H), 10.6 (d, 1H). FABMS 394 (M+H), 400.3 (M + Li).

Example 45

2S-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]butanedioic acid.



3S-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-4-(methoxymethylamino)-4-oxobutanoic acid (1.6g) from Example 44 was taken up in 300 mL water made acidic (pH 2) with trifluoroacetic acid and heated

at 60°C for several hours. The product was isolated by preparative RPHPLC and lyophilized to give desired product (0.84 g). ¹H NMR (300 MHz, d₆ DMSO) δ 2.55 (bm, 6H), 4.5 (m, 1H), 7.8 (s, 4H), 8.2 (d, 1H), 9.15 (bs, 4H), 10.4 (d, 1H), FABMS 351.3 (MH⁺).

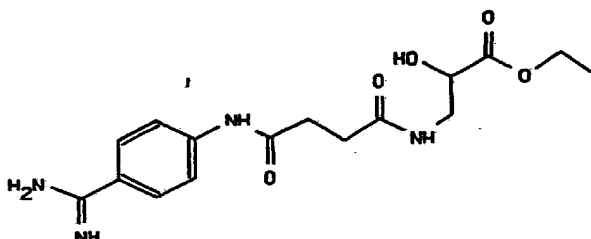
Elemental Analysis

Required for

C ₂₃ H ₂₇ N ₄ O ₇ ·F ₃ C ₂ O ₂ H·H ₂ O:	C 52.27	H 5.15	N 10.78
Found:	C 52.08	H 4.84	N 10.45

EXAMPLE 46

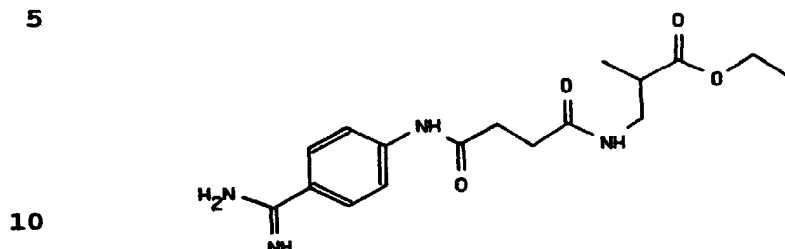
Ethyl 3-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-2-hydroxy-propanoate



The title compound was prepared in the manner of Example 1 substituting ethyl 3-amino-2-hydroxypropanoate for D,L-3-amino-3-phenylpropionic acid of Example 1 to afford the title compound. The product was verified by ¹³C NMR (CD₃OD) δ 15.6, 29.1, 31.9, 42.1, 60.8, 69.1, 119.2, 122.2, 128.8, 144.3, 166.3, 172.0, 172.9, 173.6; Fast Atom Bombardment Mass Spectrometry (MH⁺)=351.

EXAMPLE 47

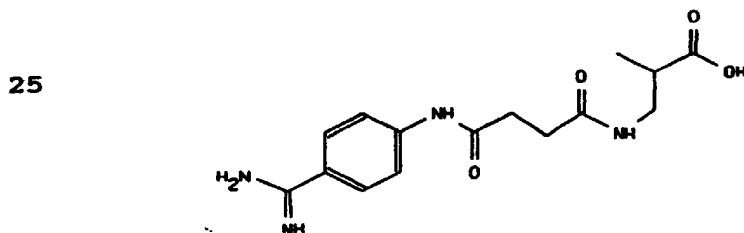
Ethyl 3-[[4-[[4-(aminoiminomethyl)phenyl]
amino]-1,4-dioxobutyl]amino]-2-methylpropanoate



The title compound was prepared in the manner
of Example 1 substituting ethyl 3-aminoisobutanoate for
15 D,L-3-amino-3-phenylpropionic acid of Example 1 to
afford the title compound. Fast Atom Bombardment mass
spectrometry (MH^+)=349.

EXAMPLE 48

20 3-[[4-[[4-(aminoiminomethyl)phenyl]
amino]-1,4-dioxobutyl]amino]-2-methylpropanoic acid



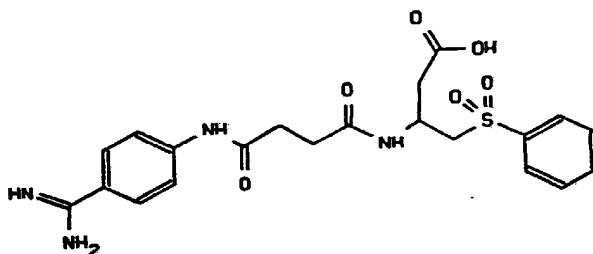
30

The title compound was prepared by treating
the final product of the previous example with porcine
liver esterase in the manner of Example 29. The product
was purified by reverse phase HPLC using the conditions
35 of Example 1 to afford the title compound. The product
was verified by Fast Atom Bombardment mass spectrometry
(MH^+)=321.

EXAMPLE 49

3-[[4-[[4-(aminoiminomethyl)phenyl]-
amino]-1,4-dioxobutyl]amino]-4-phenylsulfonyl butanoic
5 acid.

10



15 Step 1 Preparation of 3-amino-4-phenylsulfonyl
butanoic acid.

Thiophenol (5 g, 45 mmol) was added to K_2CO_3 (9.4 g, 67.5 mmol) in dry DMF (100 ml). To this mixture was added methyl 4-chloroacetoacetate (6.8 g, 45 mmol). The
20 progress of the reaction was monitored by tlc (ethyl acetate/hexane 30%). After complete reaction (~ 3 h) 10% aq. HCl was added and the ketoester was extracted into ether to leave 10 g of an amber oil. The crude ketoester was dissolved in methanol (200 ml).
25 To this solution ammonium formate (28 g, 670 mmol) was added followed by $NaBH_3CN$ (2.8 g, 67 mmol) and the solution was stirred for 24 h. The methanol was removed under reduced pressure to leave a solid mass which was diluted with methylene chloride. The excess ammonium
30 formate was removed and the filtrate concentrated to leave crude 3-amino ester. The amino ester was extracted into 10% aq. HCl and extracted with ether. The ether extracts were discarded and the aq. layer made basic with aq. NaOH. The product purified by RPHPLC
35 (0.05% TFA water/acetonitrile) to result in 3.5 g of a white solid: 1H NMR (d_6 -DMSO) δ 2.7 (2H), 3.2 (2H), 3.5 (m, 1H), 7.33 (m, 5H), 8.02 (bs, 2H).

Step 2 Preparation of 3-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-4-phenylsulfonyl butanoic acid.

- 4-[[4-(aminoiminomethyl)phenyl]amino]-4-oxobutanoic acid
5 hydrochloride prepared in Example 1, Step 1 (3.0 g, 11.3 mmol) was added to dry DMF (200 ml) followed by N-methylmorpholine (1.2 g, 11.3 mmol) and isobutyl chloroformate (1.5 g, 17 mmol) at 25°C. The mixture was stirred for 5 min. 3-Amino-4-phenylsulfonyl butanoic
10 acid (4.0 g, 11.3 mmol) was added followed by triethylamine (2.7 g) and dimethylaminopyridine. After 1 hr, the solvent was removed under reduced pressure and the solid mass was dissolved into a mixture of acetic acid:H₂O:H₂O₂ 30% (2:2:1) and stirred at 25°C for 48 hr.
15 The reaction mixture was concentrated in vacuo and the residue purified by reverse phase chromatography (.05% TFA water/acetonitrile) to result in 3.5 g of a white solid: ¹H NMR (d6-DMSO) 2.25 (m, 2H), 2.53 (m, 4H), 3.55 (m, 2H), 4.33 (m, 1H), 7.44 (m, 5H), 7.79 (s, 4H), 7.99 (d, 1H, J = 8.1 Hz), 9.1 (bs, 2H), 9.19 (bs, 2H), 10.42 (s, 1H); MS
20 (FAB) m/z 461.2 (MH⁺).

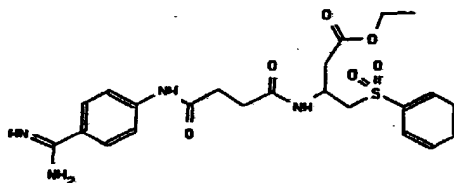
Elemental Analysis

Required for

- 25 C₂₁H₂₄N₄O₆ · F₃C₂O₂H · H₂O: C 46.27 H 4.64 N 9.38
Found: C 46.01 H 4.21 N 9.12

EXAMPLE 50

Ethyl-3-[[4-[[4-(aminoiminomethyl)phenyl]-
amino]-1,4-dioxobutyl]amino]-4-phenylsulfonyl butanoate.



Ethyl-3-[[4-[[4-(aminoiminomethyl)phenyl]-
15 amino]-1,4-dioxobutyl]amino]-4-phenylsulfonyl butanoic
acid (1.2 g) from Example 49 was suspended in dry
ethanol (40 ml), and 4N HCl in dioxane (20 ml) was
added. The progress of the reaction was monitored by
RPHPLC. After 2 hours, the solvent was removed by
20 rotary evaporator to leave a white solid which was
purified by reverse phase chromatography
(water/acetonitrile) to result in 1.1 g of a white
solid: ¹H NMR (d₆-DMSO) δ 1.1 (t, 3H, J=7.5 Hz), 2.25 (m,
2H), 2.53 (m, 4H), 3.55 (m, 2H), 4.0 (q, 2H, J=7.5 Hz)
25 4.33 (m, 1H), 7.44 (m, 5H), 7.79 (s, 4H), 7.99 (d,
1H, J=8.1 Hz), 9.1 (bs, 2H), 9.19 (bs, 2H), 10.42 (s,
1H); MS (FAB) m/z 489.3 (MH⁺).

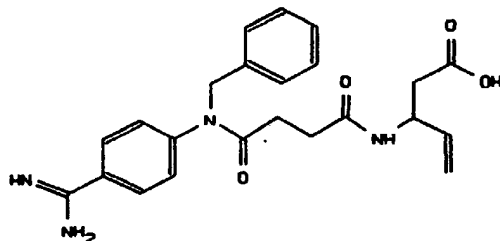
Elemental Analysis

Required for

30 C₂₃H₂₈N₄O₆S.F₃C₂O₂H . H₂O: C 48.38 H 5.03 N 9.03
Found: C 48.72 H 4.74 N 9.03

EXAMPLE 51

3-[[4-[[4-(aminoiminomethyl)phenyl](phenylmethyl)amino]-1,4-dioxobutyl]aminobutyl]amino]-4-pentenoic acid.



The ethyl ester of the title compound was prepared in the manner of Example 1 substituting 4-[[4-(aminoiminomethyl)phenyl](phenylmethyl)amino]-4-oxobutanoic acid hydrochloride for 4-[[4-(aminoiminomethyl)phenyl]amino]-4-oxobutanoic acid hydrochloride. The ethyl ester was cleaved by treatment with porcine liver esterase in the manner of Example 29. The product was purified by reverse phase HPLC using the conditions of Example 1 to afford the title compound. The product was verified by ^{13}C NMR (CD_3OD) δ 29.4, 30.5, 38.6, 52.4, 114.3, 127.2, 128.0, 128.2, 129.0, 129.0, 136.7, 136.9, 147.0, 166.1, 172.2, 172.3, 172.8; Chemical Ionization Mass Spectrometry (MH^+)=423.

Anal. Calcd. for

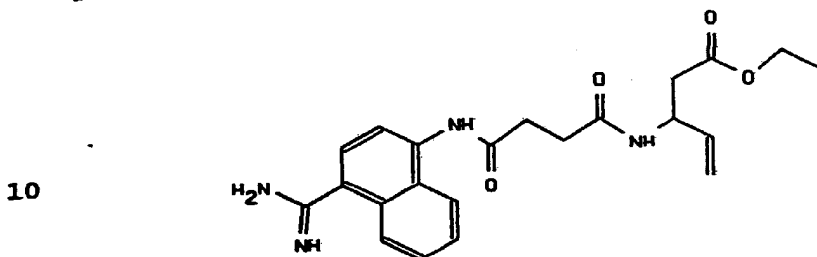
$\text{C}_{23}\text{H}_{26}\text{N}_4\text{O}_4 \cdot 1.0 \text{ CF}_3\text{CO}_2\text{H} \cdot 2.0 \text{ H}_2\text{O}$: C 52.45 H 5.46 N 9.79

Found:

C 52.53 H 5.25 N 9.60

EXAMPLE 52

Ethyl 3-[[[4-[[4-(aminoiminomethyl)naphthyl]
(phenylmethyl)-amino]-1,4-dioxobutyl]amino]-4-
5 pentenoate.



15 The title compound was prepared in the manner of Example 1 substituting 4-[[4-(aminoiminomethyl)naphthyl]amino]-4-oxobutanoic acid hydrochloride for 4-[[4-(aminoiminomethyl)-3-phenyl]amino]-4-oxobutanoic acid hydrochloride and using
20 ethyl 3-amino-4-pentenoate. The product was purified by reverse phase HPLC using the conditions of Example 1 to afford the title compound. The product was verified by
25 ^{13}C NMR (CD_3OD) δ 13.2, 30.5, 31.3, 39.0, 48.5, 60.4, 114.6, 120.3, 123.1, 123.9, 124.8, 126.6, 127.0, 128.0, 128.1, 130.3, 136.8, 137.4, 167.6, 171.0, 172.6, 173.0;
Chemical Ionization Mass Spectrometry (MH^+)=411

Anal. Calcd. for

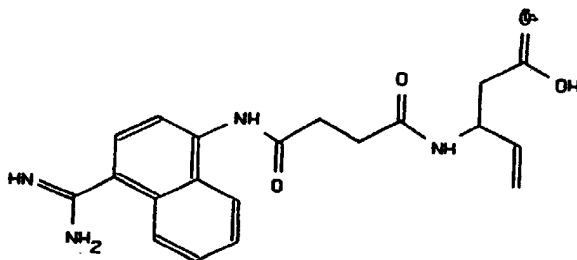
$\text{C}_{22}\text{H}_{26}\text{N}_4\text{O}_4 \cdot 1.25 \text{ CF}_3\text{CO}_2\text{H}$: C 53.21 H 4.97 N 10.13

30 Found:

C 53.23 H 5.01 N 10.24

EXAMPLE 53

3-[[4-[[4-(aminoiminomethyl)naphtyl](phenylmethyl)-amino]-1,4-dioxobutyl]amino]-4-pentenoic acid.



The title compound was prepared by treating the final product of the previous example with porcine liver esterase in the manner of Example 29. The product was purified by reverse phase HPLC using the conditions of Example 1 to afford the title compound. The product was verified by ^{13}C NMR (CD_3OD) δ 30.4, 31.2, 38.6, 48.2, 114.3, 120.3, 123.0, 123.8, 126.5, 126.9, 127.9, 128.0, 130.2, 136.8, 137.3, 167.5, 172.5, 172.8, 172.9; Fast Atom Bombardment Mass Spectrometry (MH^+) = 383.

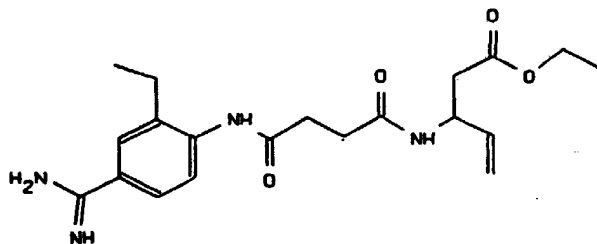
Anal Calcd for

$\text{C}_{20}\text{H}_{22}\text{N}_4\text{O}_4 \cdot 1.25 \text{ CF}_3\text{CO}_2\text{H}$: C 51.48 H 4.46 N 10.67

Found: C 51.05 H 4.41 N 10.66

EXAMPLE 54

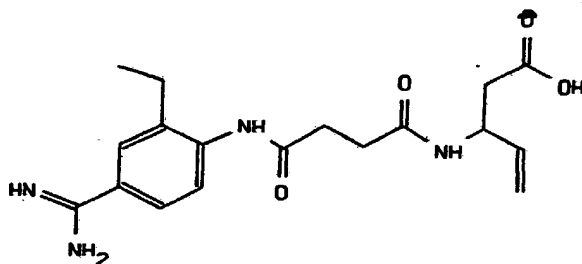
Ethyl 3-[[4-[[4-(aminoiminomethyl)-2-ethylphenyl]amino]-1,4-dioxobutyl]amino]-4-pentenoate



The title compound was prepared in the manner of Example 1 substituting 4-[[4-(aminoiminomethyl)-2-ethylphenyl]amino]-4-oxobutanoic acid hydrochloride for 4-[[4-(aminoiminomethyl)phenyl]amino]-4-oxobutanoic acid hydrochloride and using ethyl 3-amino-4-pentenoate. The product was purified by reverse phase HPLC using the conditions of Example 1 to afford the title compound. The product was verified by ^{13}C NMR (CD_3OD) δ 12.8, 13.1, 23.7, 30.5, 31.3, 38.9, 48.4, 60.4, 114.5, 124.6, 125.2, 125.6, 128.1, 136.8, 138.0, 140.9, 166.8, 171.0, 171.1, 172.5; Chemical Ionization Mass Spectrometry (MH^+) = 389. Anal Calcd for $\text{C}_{20}\text{H}_{28}\text{N}_4\text{O}_4 \cdot 1.0 \text{ CF}_3\text{CO}_2\text{H} \cdot .25 \text{ H}_2\text{O}$: C 52.12 H 5.86 N 11.04 Found: C 52.04 H 6.15 N 11.15

EXAMPLE 55

3-[[4-[[4-(aminoiminomethyl)-2-ethylphenyl]amino]-1,4-dioxobutyl]amino]-4-pentenoic acid.



The title compound was prepared by treating the final product of the Example 54 with porcine liver esterase in the manner of Example 29. The product was purified by RPHPLC using the conditions of Example 1 to afford the title compound. The product was verified by ^{13}C NMR (CD_3OD) δ 11.9, 22.9, 29.6, 30.4, 37.8, 47.3, 113.5, 123.6, 124.4, 124.7, 127.3, 136.0, 137.4, 140.1, 166.5, 171.7, 171.8, 172.5; Chemical Ionization Mass Spectrometry (MH^+) = 361.

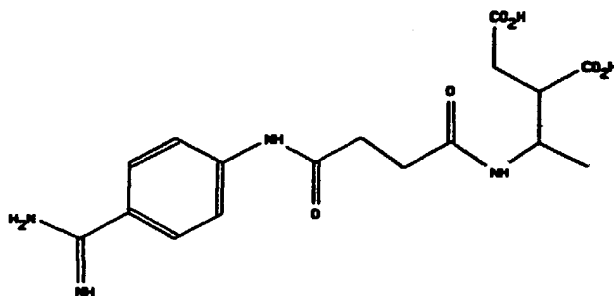
Anal Calcd for

$C_{18}H_{24}N_4O_4 \cdot 1.25 CF_3CO_2H \cdot 0.25 H_2O$: C 48.52 H 5.11 N 11.04

Found: C 48.31 H 5.04 N 10.95

EXAMPLE 56

2-[1-[4-[4-(aminoiminomethyl)phenyl]-amino]-1,4-dioxobutyl] amino]-ethyl]succinic acid.



Dimethyl-3-[[4-[[4-(aminoiminomethyl)phenyl]-amino]-1,4-dioxobutyl]amino-butyl]amino]-2-(aminoethyl) succinate prepared in Example 15a, Step 1 (600 mg) was added to water/acetonitrile (20 ml) followed by lithium hydroxide (150 mg) at 25°C. The mixture was stirred for 30 min. The course of the reaction was monitored by RPHPLC. After satisfactory product was formed the reaction was neutralized with TFA and purified by reverse phase chromatography (0.05% TFA

water/acetonitrile) to result in 250 mg of a white solid: 1H NMR (d_6 -DMSO) δ 0.99 (d, 3H, $J=7.1$ Hz), 2.44 (m, 2H), 2.55 (m, 4H), 3.14 (m, 1H), 4.15 (m, 1H), 7.78 (s, 4H), 7.92 (d, 1H, $J=7.7$ Hz), 8.84 (bs, 2H), 9.16 (bs, 2H), 10.25 (s, 1H); MS (FAB) m/z 379.1 (MH^+)

Elemental Analysis

Required for

$C_{17}H_{22}N_4O_6 \cdot F_3C_2O_2H \cdot H_2O$: C 43.18 H 5.11 N 10.60

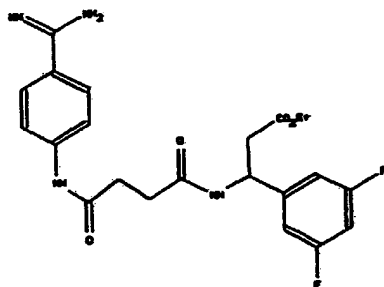
Found: C 43.25 H 4.62 N 10.17

EXAMPLE 57

Ethyl β -[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-(3,5-difluorophenyl)propanoate.

5

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Step 1 4-[[4-(aminoiminomethyl)phenyl]-amino]-4-oxobutanoic acid hydrochloride prepared in Example 1, followed by N-methylmorpholine (1.7 g, 18.5 mmol) and isobutyl chloroformate (2.8 g, 17 mmol) at 25°C. The mixture was stirred for 5 min. Ethyl-3-amino-3-(3,5-difluorophenyl)propanoate (3.0 g, 18.5 mmol) was added followed by dimethylaminopyridine. After 1 h, the solvent was removed under reduced pressure and the product purified by reverse phase chromatography (.05% TFA water/acetonitrile) to result in 2.0 g of a white solid: ^1H NMR (d_6 -DMSO) δ 2.57 (t, 2H, $J=7.31$ Hz), 2.07 (t, 2H, $J=7.1$ Hz), 3.47 (t, 2H, $J=7.0$ Hz), 3.5 (s, 6H), 3.51 (m, 1H), 7.79 (s, 4H), 8.1 (t, 1H, $J=7.1$ Hz), 8.7 (bs, 2H), 9.09 (bs, 2H), 10.32 (s, 1H); MS (FAB) m/z 379.0 (MH^+).

Elemental Analysis

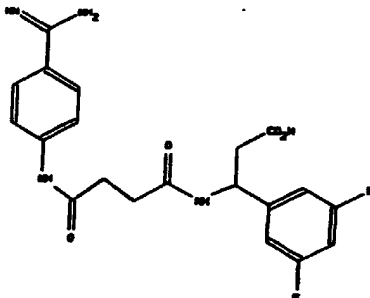
30 Required for

$\text{C}_{17}\text{H}_{22}\text{N}_4\text{O}_6 \cdot \text{F}_3\text{C}_2\text{O}_2\text{H} \cdot \text{H}_2\text{O}$:

Found:

C 45.50	H 4.72	N 11.18
C 45.20	H 4.66	N 11.17

β -[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-(3,5-difluorophenyl)propanoate



Required for

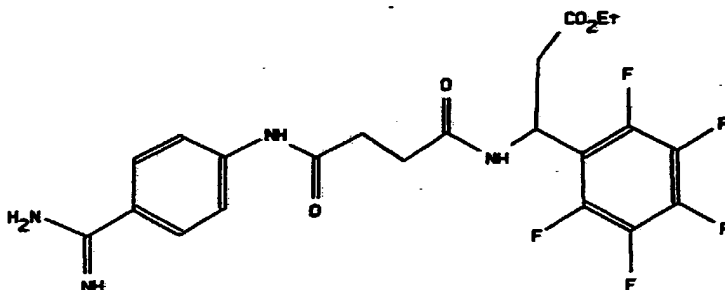
30	$\text{C}_{16}\text{H}_{20}\text{N}_4\text{O}_6 \cdot \text{F}_3\text{C}_2\text{O}_2\text{H} \cdot \text{H}_2\text{O}:$	C 43.54	H 4.64	N 11.13
	Found:	C 43.40	H 4.52	N 11.18

EXAMPLE 59

Ethyl β [[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-(pentafluoro-phenyl)propanoate.

5

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4-[[4-(aminoiminomethyl)phenyl]amino]-4-oxobutanoic acid
 15 hydrochloride prepared in Example 1, Step 1 (5.0 g, 18.5 mmol) was added to dry DMF (250 ml) followed by N-methylmorpholine (1.7 g, 18.5 mmol) and isobutyl chloroformate (2.8 g, 17 mmol) at 25 C. The mixture was stirred for 5 min. Ethyl 3-amino-3-(3,5-
 20 difluorophenyl)propanoate (3.0 g, 18.5 mmol) was added followed by dimethylaminopyridine. After 1 h, the solvent was removed under reduced pressure and the product purified by reverse phase chromatography (.05% TFA water/acetonitrile) to result in 2.0 g of a white
 25 solid: ^1H NMR (d_6 -DMSO) δ 2.57 (t, 2H, $J=7.3$ Hz), 2.07 (t, 2H, $J=7.1$ Hz), 3.47 (t, 2H, $J=7.0$ Hz), 3.5 (s, 6H), 3.51 (m, 1H), 7.79 (s, 4H), 8.1 (t, 1H, $J=7.1$ Hz), 8.7 (bs, 2H), 9.09 (bs, 2H), 10.32 (s, 1H); MS (FAB) m/z 379.0 (MH^+).

30 Elemental Analysis

Required for

 $\text{C}_{17}\text{H}_{22}\text{N}_4\text{O}_6 \cdot \text{F}_3\text{C}_2\text{O}_2\text{H} \cdot \text{H}_2\text{O}$:

C 45.50 H 4.72 N 11.18

Found:

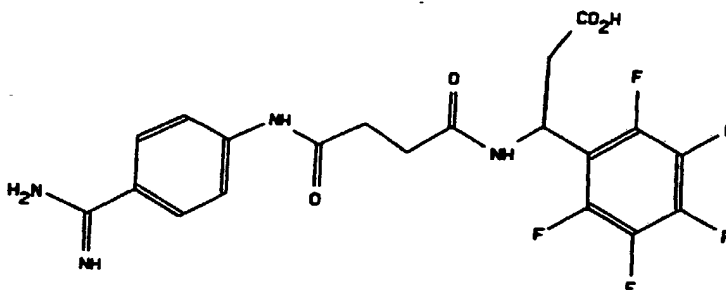
C 45.20 H 4.66 N 11.17

EXAMPLE 60

β [[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-(pentafluorophenyl)propanoic acid.

5

10



Ethyl β [[4-[[4-(aminoiminomethyl)phenyl]-
15 amino]-1,4-dioxobutyl]amino]-(pentafluorophenyl)propanoate prepared in Example 59 (600 mg) was added to water/acetonitrile (20 ml) followed by lithium hydroxide (100 mg) at 25°C. The mixture was stirred for 30 min. The course of the
20 reaction was monitored by RPHPLC. After satisfactory acid was formed the reaction was neutralized with TFA and purified by reverse phase chromatography (water/acetonitrile) to result in 620 mg of a white solid: ^1H NMR (d_6 -DMSO) δ 2.38 (t, 2H, $J=7.3$ Hz), 2.44 (d, 2H, $J=6.4$ Hz), 2.56 (t, 2H, $J=7.3$ Hz), 4.32 (m, 1H), 7.78 (s, 4H), 7.99 (d, 1H, $J=8.1$ Hz), 8.92 (bs, 2H), 9.16 (bs, 2H), 10.39 (s, 1H); MS (FAB) m/z 365.2 (MH^+).

Elemental Analysis

Required for

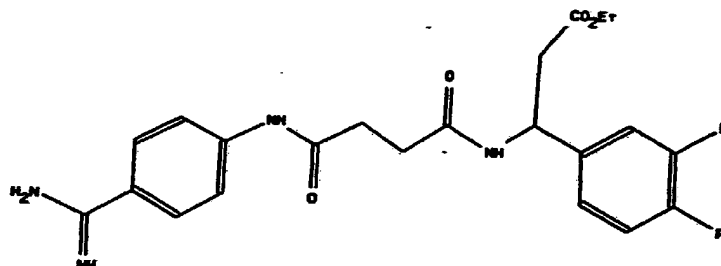
30 $\text{C}_{16}\text{H}_{20}\text{N}_4\text{O}_6 \cdot \text{F}_3\text{C}_2\text{O}_2\text{H} \cdot \text{H}_2\text{O}$: C 43.54 H 4.64 N 11.13
Found: C 43.40 H 4.52 N 11.18

EXAMPLE 61

Ethyl [[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-(3,4-difluorophenyl)propanoate.

5

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4-[[4-(aminoiminomethyl)phenyl]amino]-4-oxobutanoic acid hydrochloride prepared in Example 1, Step 1 (5.0 g, 18.5 mmol) was added to dry DMF (250 ml) followed by N-methylmorpholine (1.7 g, 18.5 mmol) and isobutyl chloroformate (2.8 g, 17 mmol) at 25°C. The mixture was stirred for 5 min. Ethyl 3-amino-3-(3,5-difluorophenyl)propanoate (3.0 g, 18.5 mmol) was added followed by dimethylaminopyridine. After 1 h, the solvent was removed under reduced pressure and the product purified by reverse phase chromatography (water/acetonitrile) to result in 2.0 g of a white solid: ¹H NMR (d₆-DMSO) δ 2.57 (t, 2H, J=7.3 Hz), 2.07 (t, 2H, J=7.1 Hz), 3.47 (t, 2H, J= 7.0 Hz), 3.5 (s, 6H), 3.51 (m, 1H), 7.79 (s, 4H), 8.1 (t, 1H, J=7.1 Hz), 8.7 (bs, 2H), 9.09 (bs, 2H), 10.32 (s, 1H); MS (FAB) m/z 379.0 (MH⁺).

Elemental Analysis
Required for

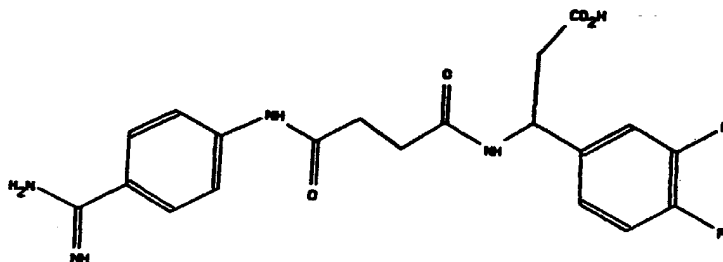
C ₁₇ H ₂₂ N ₄ O ₆ · F ₃ C ₂ O ₂ H · H ₂ O:	C 45.50	H 4.72	N 11.18
Found:	C 45.20	H 4.66	N 11.17

EXAMPLE 62

β [[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-(3,4-difluorophenyl) propanoic acid.

5

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Ethyl [[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-(3,4-difluorophenyl)propanoate prepared in Example 61 (700 mg) was added to water/acetonitrile (20 ml) followed by lithium hydroxide (100 mg) at 25°C. The mixture was stirred for 30 min. The course of the reaction was monitored by RPHPLC. After satisfactory acid was formed the reaction was neutralized with TFA and purified by reverse phase chromatography (water/acetonitrile) to result in 620 mg of a white solid: ^1H NMR (d_6 -DMSO) δ 2.38 (t, 2H, $J=7.3$ Hz), 2.44 (d, 2H, $J=6.4$ Hz), 2.56 (t, 2H, $J=7.3$ Hz), 4.32 (m, 1H), 7.78 (s, 4H), 7.99 (d, 1H, $J=8.1$ Hz), 8.92 (bs, 2H), 9.16 (bs, 2H), 10.39 (s, 1H); MS (FAB) m/z 365.2 (MH^+).

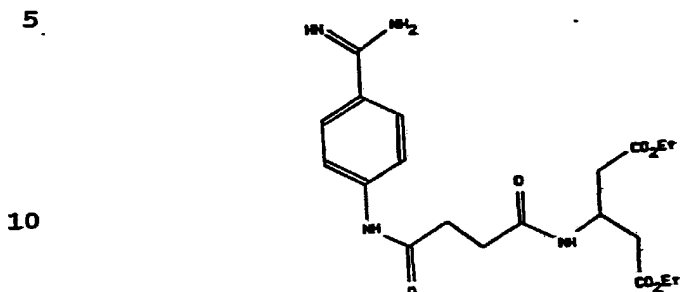
Elemental Analysis

Required for

30	$\text{C}_{16} \text{H}_{20} \text{N}_4 \text{O}_6 \cdot \text{F}_3 \text{C}_2 \text{O}_2 \text{H} \cdot \text{H}_2 \text{O}$:	C 43.54	H 4.64	N 11.13
	Found:	C 43.40	H 4.52	N 11.18

EXAMPLE 63

Diethyl 3-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]pentanedioate.



4-[[4-(aminoiminomethyl)phenyl]amino]-4-oxobutanoic acid hydrochloride prepared in Example 1, Step 1 (4.6 g, 17 mmol) was added to dry DMF (225 ml) followed by N-methylmorpholine (1.2 g, 17 mmol) and isobutyl chloroformate (2.3 g, 17 mmol) at 25°C. The mixture was stirred for 5 min. Dimethyl-3-aminoglutarate (3.0 g, 17 mmol) was added followed by dimethylaminopyridine. After 1 h, the solvent was removed under reduced pressure and the product purified by reverse phase chromatography (.05% TFA water/acetonitrile) to result in 3.5 g of a white solid:

25 ¹H NMR (d₆-DMSO) δ 1.37 (t, 2H, J=7.8 Hz), 2.55 (m, 2H), 2.57 (t, 2H, J=7.1 Hz), 3.57 (s, 6H), 4.22 (q, 4H, J= 7.8 Hz), 4.35 (m, 1H), 7.79 (s, 4H), 7.99 (d, 1H, J=8.1 Hz), 9.1 (bs, 2H), 9.19 (bs, 2H), 10.42 (s, 1H); MS (FAB) m/z 393.2 (MH⁺).

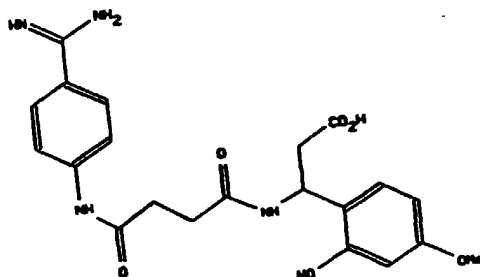
30 **Elemental Analysis**

Required for

C ₁₈ H ₂₄ N ₄ O ₆ · F ₃ C ₂ O ₂ H · H ₂ O:	C 47.42	H 4.91	N 11.14
Found:	C 47.12	H 4.97	N 10.99

EXAMPLE 64

3-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-3-(2-hydroxy-4-methoxyphenyl)propanoic acid.



4-[[4-(aminoiminomethyl)phenyl]amino]-4-oxobutanoic acid hydrochloride prepared in Example 1, Step 1 (4.6 g, 17 mmol) was added to dry DMF (225 ml) followed by N-methylmorpholine (1.2 g, 17 mmol) and isobutyl chloroformate (2.3 g, 17 mmol) at 25°C. The mixture was stirred for 5 min. Amino methoxy coumarin (3.0 g, 17 mmol) was added followed by dimethylaminopyridine. After 1 h, the solvent was removed under reduced pressure and the product purified by reverse phase chromatography (water/acetonitrile) to result in 3.5 g of a white solid: ¹H NMR (d₆-DMSO) δ 2.37 (t, 2H, J=7.3 Hz), 2.55 (m, 2H), 2.57 (t, 2H, J=7.1 Hz), 3.57 (s, 6H), 4.35 (m, 1H), 7.79 (s, 4H), 7.99 (d, 1H, J=8.1 Hz), 9.1 (bs, 2H), 9.19 (bs, 2H), 10.42 (s, 1H); MS (FAB) m/z 393.2 (MH⁺).

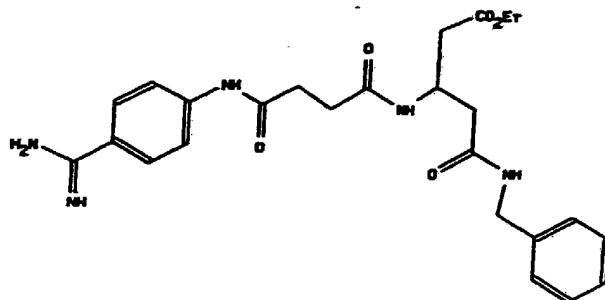
Elemental Analysis

Required for

C ₁₈ H ₂₄ N ₄ O ₆ · F ₃ C ₂ O ₂ H · H ₂ O:	C 47.42	H 4.91	N 11.14
Found:	C 47.12	H 4.97	N 10.99

EXAMPLE 65

Ethyl 3-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-5-oxo-5[(phenylmethyl)amino]pentanoate



Step 1 4-[[4-(aminoiminomethyl)phenyl]amino]-4-oxobutanoic acid hydrochloride prepared in Example 1, Step 1 (5.0 g, 18.5 mmol) was added to dry DMF (250 ml) followed by N-methylmorpholine (1.7 g, 18.5 mmol) and isobutyl chloroformate (2.8 g, 17 mmol) at 25°C. The mixture was stirred for 5 min. Ethyl 3-amino-3-(3,5-difluorophenyl)propanoate (3.0 g, 18.5 mmol) was added followed by dimethylaminopyridine. After 1 h, the solvent was removed under reduced pressure and the product purified by reverse phase chromatography (.05% TFA water/acetonitrile) to result in 2.0 g of a white solid: ¹H NMR (d₆-DMSO) δ 2.57 (t, 2H, J=7.3 Hz), 2.07 (t, 2H, J=7.1 Hz), 3.47 (t, 2H, J= 7.0 Hz), 3.5 (s, 6H), 3.51 (m, 1H), 7.79 (s, 4H), 8.1 (t, 1H, J=7.1 Hz), 8.7 (bs, 2H), 9.09 (bs, 2H), 10.32 (s, 1H); MS (FAB) m/z 379.0 (MH⁺).

Elemental Analysis

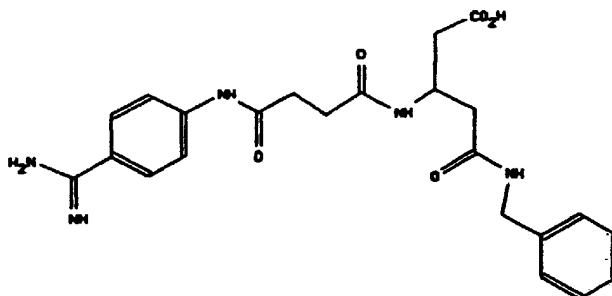
Required for

C ₁₇ H ₂₂ N ₄ O ₆ · F ₃ C ₂ O ₂ H · H ₂ O:	C 45.50	H 4.72	N 11.18
Found:	C 45.20	H 4.66	N 11.17

EXAMPLE 66

3-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-
 5 dioxobutyl]amino]-5-oxo-5-[(phenylmethyl)amino]pentanoic
 acid

10



15

The final product of Example 65 (720 mg) was added to water/acetonitrile (20 ml) followed by lithium hydroxide (100 mg), at 25°C. The mixture was stirred for 30 min. The course of the reaction was monitored by RPHPLC. After satisfactory acid was formed the reaction
 20 was neutralized with TFA and purified by reverse phase chromatography (.05% TFA water/acetonitrile) to result in 620 mg of a white solid: ¹H NMR (d₆-DMSO) δ 2.38 (t, 2H, J=7.3 Hz), 2.44 (d, 2H, J=6.4 Hz), 2.56 (t, 2H, J=7.3 Hz), 4.32 (m, 1H), 7.78 (s, 4H), 7.99 (d, 1H, J=8.1
 25 Hz), 8.92 (bs, 2H), 9.16 (bs, 2H), 10.39 (s, 1H); MS (FAB) m/z 365.2 (MH⁺).

Elemental AnalysisRequired for

C₁₆ H₂₀ N₄ O₆ · F₃C₂O₂H · H₂O: C 43.54 H 4.64 N 11.13

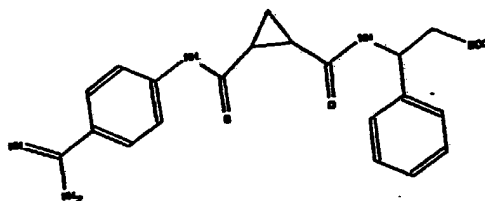
30 Found: C 43.40 H 4.52 N 11.18

Example 67

t-Butyl-
β-[[[2-[[[4-(aminoiminomethyl)phenyl]amino]carbonyl]
cyclopropyl]carbonyl]amino]-phenylpropanoate

5

10



Step 1 Preparation of [2-[ethoxycarbonyl]cyclopropyl]
15 carboxylic acid.

Diethyl cyclopropyl carboxylate (50 g, 0.268 mol; trans isomer from Aldrich) in 100 mL ethanol was added to a solution of 10 g LiOH (0.238 mol) in 100 mL H₂O. After 5 min stirring, a yellow homogeneous mixture was observed
20 and stirring continued for 24 h at 25°C. The crude reaction mixture was partitioned between ethyl acetate and water (pH = 9). Then the aqueous layer was made acidic (pH = 2) and extracted with ethyl acetate. The ethyl acetate extract was dried (MgSO₄) and concentrated
25 to give 23 g of the desired mono acid as a solid (mp 46°C).

Step 2 Preparation of

2-[[[4-(aminoiminomethyl)phenyl]amino]carbonyl]cyclopropyl-carboxylic acid.

30 To 6.0 g (0.038 mol) ethyl trans -2-carboxyl cyclopropanecarboxylate dissolved in 100 mL anhydrous DMF and 10 mL anhydrous pyridine was added 4.82 g (4.92 mL, 0.040 mol) trimethylacetyl chloride and a catalytic amount of DMAP. After about one hour, 9.49 g
35 (0.046 mol) benzamidine was added and allowed to react under argon at room temperature overnight. The volatiles were removed by vacuum on a rotavap at 55°C

until a viscous oil was obtained. The residue was dissolved in water (100 mL) and the pH adjusted to 12 by addition of aqueous LiOH. After stirring overnight a precipitate formed. The pH was adjusted to 7 by
5 addition of dilute aqueous HCl and the solids filtered and dried in a desiccator to give 5.0 g (53 %) of the desired zwitterion product. This material was converted to the hydrochloride salt by contacting with 4 N HCl in dioxane (100 mL) for several hours. The resulting solid
10 was collected, washed with diethyl ether and dried.

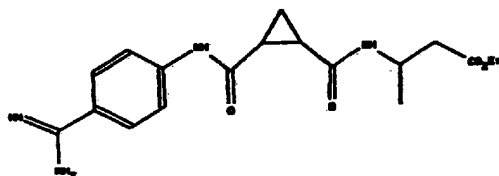
Step 2 t-Butyl-

β (S)-[[[2-[[[4-(aminoiminomethyl)phenyl]amino]carbonyl]cyclopropyl]carbonyl]amino]-phenylpropanoate
15 0.90 g (0.0036 mol) of
2-[[[4-(aminoiminomethyl)phenyl]amino]carbonyl]cyclopropyl-carboxylic acid was treated with 50 mL dry DMF and the solvent removed at 55°C. To the remaining solid was added 60 mL DMF, 6 mL pyridine and 0.43 g
20 (0.44 mL, 0.0036 mol) trimethylacetyl chloride. After 0.5 hr at room temperature the reaction mixture was heated at 55°C for 0.5 hr then 0.93 g (0.004 mol) tert - butyl-3-amino-3-phenyl propionate and 0.36 g (0.40 mL , 0.004 mol) N-methylmorpholine were added and
25 the reaction allowed to proceed overnight. At this point volatiles were removed and the desired product isolated by preparative RPHPLC and lyophilized to give a diastereomeric mixture of t-butyl-
 β (S)-[[[2-[[[4-(aminoiminomethyl)phenyl]amino]carbonyl]cyclopropyl]carbonyl]amino]-3-phenylpropanoate as a
30 white powder (150 mg); ¹H NMR (300 MHz) (d₆ DMSO) δ 1.19 (m, 2H), 1.28 (s), 1.31 (s, 9H), 2.21 (m, 2H), 2.65 (d, J = 7.9 Hz), 2.66 (d, J = 7.7 Hz, 2H), 5.2 (m, 1H), 7.32 (m, 5H), 7.78 (m, 4H), 8.86 (d, 1H, J = 8.6
35 Hz), 8.95 (bs, 2H), 9.10 (bs, 2H), 10.74 (s), 10.80 (s, 1H); MS (FAB) 451.4 (MH⁺).

Example 68

ethyl β -[[[2-[[[4-(aminoiminomethyl)phenyl]amino]
carbonyl]cyclopropyl]carbonyl]amino]-butanoate,
5 isomer 1.

10



15

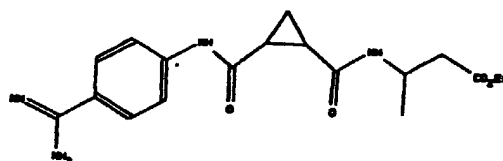
To 1.0 g (0.0035 mol)
2-[[[4-(aminoiminomethyl)phenyl]amino]carbonyl]
cyclopropyl-carboxylic acid hydrochloride in 100 mL dry
DMF was added 0.36 g (0.39 mL, 0.0035 mol) N-methyl-
20 morpholine and 0.48 g (0.46 mL, 0.0035 mol)
isobutylchloroformate. The reaction was allowed to
proceed for ten minutes at room temperature then 0.515 g
(0.576 mL , 0.0035 mol) ethyl 3-aminobutyrate was added.
The reaction was allowed to proceed overnight. The
25 volatiles were removed under vacuum at 55°C until a
viscous oil remained. The residue was dissolved in
water (60 mL) and purified by preparative RPHPLC. Two
diastereomers were obtained and separated by HPLC. The
faster eluting diastereoisomer, isomer 1, was collected
30 and lyophilized to 0.9 g of a white solid: ^1H NMR
(300 MHz) d_6 -DMSO δ 1.17 (m, 8H), 2.07 (m, 1H), 2.38
(m, 1H), 4.04 (m, 3H), 7.77 (s, 4H), 8.28 (d, J = 7.9),
8.80 (bs, 2H), 9.16 (bs , 2H), 10.78 (s , 1H); MS (FAB)
361.3 (MH $^+$).

35

Example 69

ethyl β -[[[2-[[[4-(aminoiminomethyl)phenyl]amino]
carbonyl]cyclopropyl]carbonyl]amino]butanoate,
5 isomer 2.

10



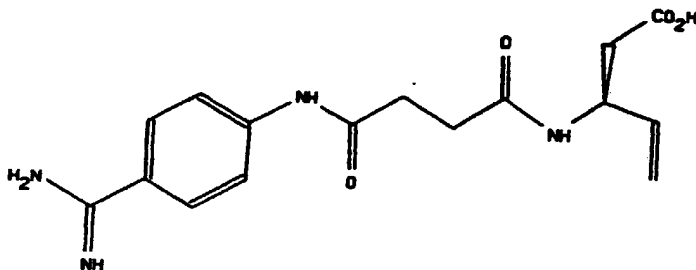
15

This compound was isolated from the reaction described
in Example 68. It was the late eluting diastereomer;
0.343 g of white fluffy solid were collected after
lyophilization; ^1H NMR 1.15 (m, 8H), 2.04 (m, 1H),
20 2.19 (m, 1H), 4.05 (m, 3H), 7.75 (s, 4H), 8.29 (d, J =
8.0 Hz), 8.80 (bs, 2H), 9.15 (bs, 2H), 10.76 (s, 1H);
MS (FAB) 361.3 (MH+).

EXAMPLE 70

25 3-(S)-3-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-
dioxobutyl]amino]-4-pentenoic acid

30



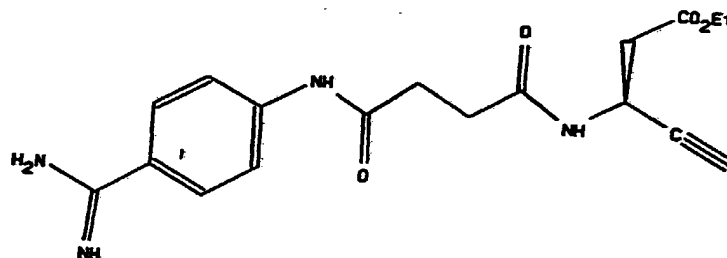
35

The title compound was prepared by treating the final product of the Example 83 with porcine liver esterase in the manner of Example 29. The product was purified by reverse phase HPLC using the conditions of Example 1 to afford the title compound.

The title compound had the identical C NMR to the racemic material of Example 33.

EXAMPLE 71

(3S)-Ethyl 3-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-4-pentynoate



The title compound was prepared in the manner of Example 38 substituting (S)-ethyl 3-amino-4-pentynoate for D,L-3-amino-4-pentynoate. The product was purified by reverse phase HPLC using the conditions for Example 1 to afford the title compound. The product had the same C NMR as Example 38. The ratio of enantiomers was determined to be 98:2 by chiral HPLC using an AGP protein column.

Anal. Calcd for $C_{20}H_{26}N_4O_4$ plus 0.2 CF_3CO_2H , 0.8 HCl and 1.0 H_2O :

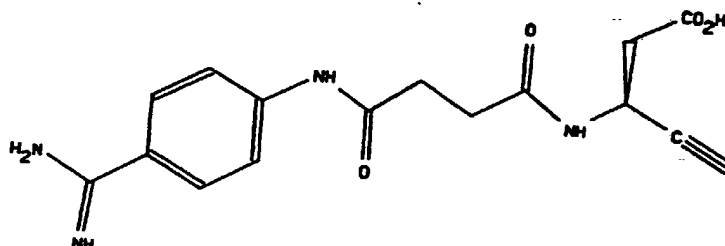
	C	51.59	H	5.88	N	13.08
Found:	C	51.68	H	5.45	N	12.89

EXAMPLE 72

(3S) -3-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-4-pentynoic acid

5

10



The title compound was prepared by treating the final
15 product of the previous example with porcine liver
esterase in the manner of Example 29. The product was
purified by reverse phase HPLC using the conditions of
Example 1 to afford the title compound. The product had
the same C NMR as Example 39. Optical Rotation $[\alpha]_D -$
20 33.7 (c 1.45, CH₃OH).

Anal. Calcd for C₁₆H₁₈N₄O₄ plus 1.85 HCl and 0.95 H₂O :

C 46.32 H 5.28 N 13.50

Found:

C 46.51 H 5.38 N 13.52

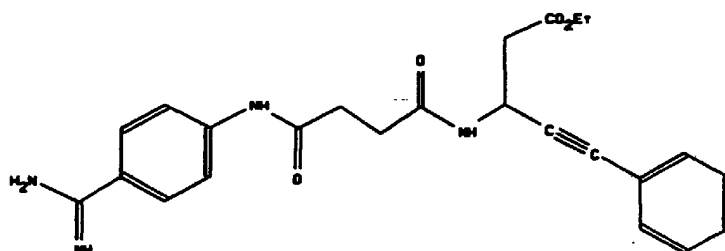
25

EXAMPLE 73

Ethyl 3-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-5-phenyl-4-pentynoate

30

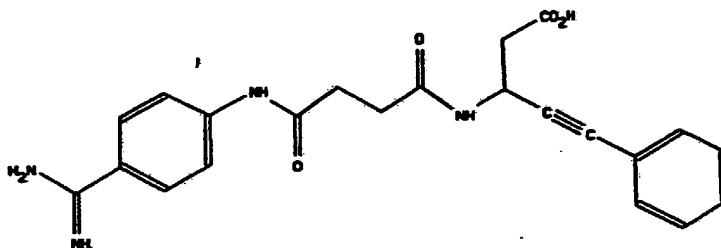
35



The title compound was prepared in the manner of Example 1 substituting ethyl 3-amino-5-phenyl-4-pentynoate for D,L-3-amino-3-phenylpropionic acid. The product was purified by reverse phase HPLC using the conditions of Example 1 to afford the title compound. The product was verified by ^{13}C NMR (CD_3OD) δ 13.6, 30.5, 31.9, 39.0, 40.5, 61.1, 83.0, 87.2, 119.7, 122.8, 122.9, 128.6, 128.8, 129.1, 131.8, 144.8, 166.7, 170.6, 172.4, 172.7. Fast Atom Bombardment Mass Spectrometry (MH^+) = 435.

EXAMPLE 74

3-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-5-phenyl-4-pentynoic Acid



The title compound was prepared by treating the final product of the previous example with porcine liver esterase in the manner of Example 29. The product was purified by reverse phase HPLC using the conditions of Example 1 to afford the title compound. The product was verified by ^1H NMR (CD_3OD) δ 2.59-2.82 (m, 3- CH_2), 5.05-5.14 (m, CHN), 7.27-7.42 (m, PhH), 7.73-7.84 (m, PhH), 8.72 (br s, NH), 9.13 (br s, NH); Fast Atom Bombardment Mass Spectrometry (MH^-) = 519.

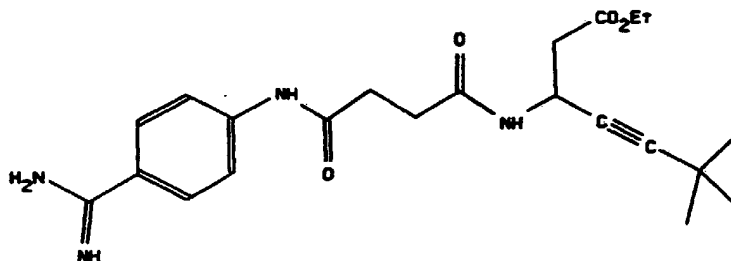
Anal. Calcd for $\text{C}_{20}\text{H}_{26}\text{N}_4\text{O}_6$ plus 1.6 $\text{CF}_3\text{CO}_2\text{H}$ and 1.0 H_2O :
C 47.48 H 5.08 N 9.55
Found: C 47.30 H 4.57 N 9.65

EXAMPLE 75

Ethyl 3-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-5,5-dimethyl-4-heptynoate

5

10



The title compound was prepared in the manner of Example 1 substituting ethyl 3-amino-5,5-dimethyl-4-heptynoate for D,L-3-amino-3-phenylpropionic acid. The product was purified by reverse phase HPLC using the conditions of Example 1 to afford the title compound. The product was verified by ^{13}C NMR (CD_3OD) δ 13.7, 30.4, 32.0, 38.6, 41.2, 61.0, 76.7, 92.7, 119.7, 122.8, 129.0, 144.7, 166.7, 170.7, 172.5. Fast Atom Bombardment Mass Spectrometry (MH^-) = 413.

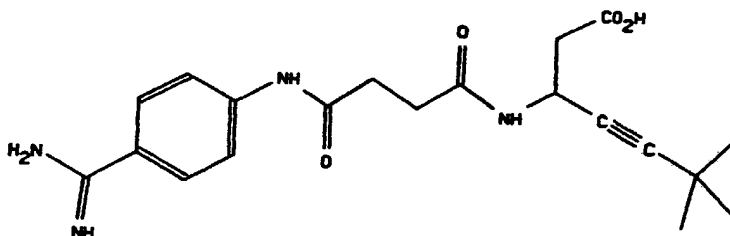
25

EXAMPLE 76

3-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-5,5-dimethyl-4-heptynoic Acid

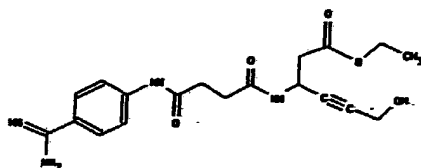
30

35



10		C	47.48	H	5.08	N	9.55
	Found	C	47.30	H	4.57	N	9.65

15 Ethyl 3-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-6-hydroxy-4-hexynoate

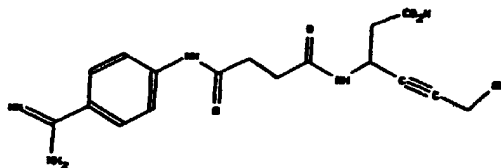


25 The title compound was prepared in the manner of Example 1 substituting ethyl 3-amino-6-hydroxy-4-hexynoate for D,L-3-amino-3-phenylpropionic acid. The product was purified by reverse phase HPLC using the conditions of

30 Example 1 to afford the title compound. The product was verified by H NMR (CD_3OD) δ 1.14 (t, J = 6Hz, CH_3), 2.57-2.78 (m, 3 CH_2), 4.09 - 4.17 (m, CH_2OH), 4.95 - 5.05 m(CHN), 7.75-7.87 (m, PhH).

EXAMPLE 78

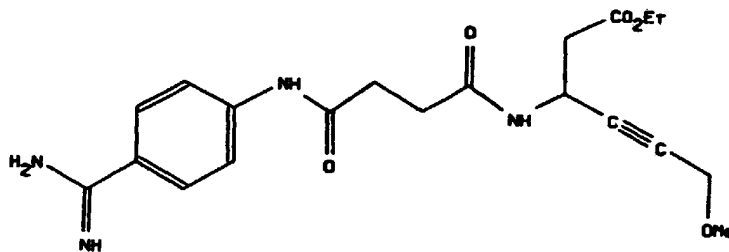
3-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-6-hydroxy-4-hexynoic Acid.



The title compound was prepared by treating the final product of the previous example with porcine liver esterase in the manner of Example 29. The product was purified by reverse phase HPLC using the conditions of Example 1 to afford the title compound. The product was verified by H NMR (CD_3OD) δ 2.57-2.78 (m, 3 CH_2), 4.16 (d, $J = 2.5$ Hz, CH_2OH), 5.00-5.09 (m, CHN), 7.75-7.87 (m, PhH).

EXAMPLE 79

Ethyl 3-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-6-methoxy-4-hexynoate

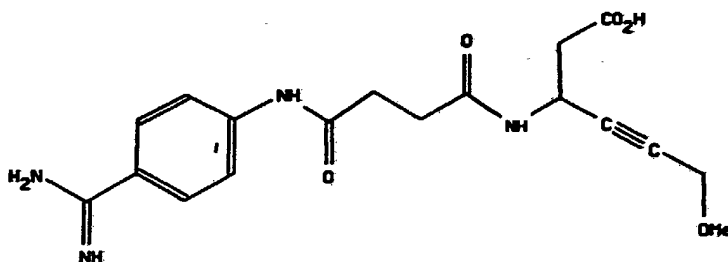


The title compound was prepared in the manner of Example 1 substituting ethyl 3-amino-6-methoxy-4-hexynoate for

D,L-3-amino-3-phenylpropionic acid. The product was purified by reverse phase HPLC using the conditions of Example 1 to afford the title compound. The product was verified by H NMR (CD_3OD) 1.25 (t, $J = 6.5$ Hz, CH_3), 2.54-2.78 (m, 3CH_2), 3.32 (s, OCH_3), 4.08 (d, $J = 2.5\text{Hz}$, CH_2OCH_3), 4.14 (q, $J = 6.5$ Hz, CH_2), 5.05-5.14 (m, CHN), 7.73-7.84 (m, PhH), 8.82 (br s, NH), 9.13 (br s, NH).

EXAMPLE 80

3-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-6-methoxy-4-hexynoic Acid.



20

The title compound was prepared by treating the final product of the previous example with porcine liver esterase in the manner of Example 29. The product was purified by reverse phase HPLC using the conditions of Example 1 to afford the title compound. The product was verified by ^{13}C NMR (CD_3OD) δ 29.1, 30.6, 37.2, 38.7, 55.5, 58.2, 77.4, 83.5, 118.4, 121.3, 127.8, 143.4, 165.7, 171.0, 171.2, 171.4.

Anal. Calcd for $\text{C}_{18}\text{H}_{22}\text{N}_4\text{O}_5$, plus 1.1 $\text{CF}_3\text{CO}_2\text{H}$, 0.65 H_2O :

C 47.61 H 4.83 N 10.99

Found:

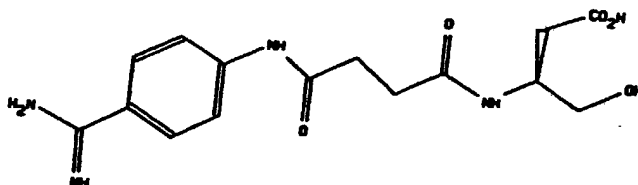
C 47.24 H 4.43 N 11.25

EXAMPLE 81

3(S)-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-4-hydroxybutanoic acid.

5

10



The lactone derivative prepared as usual by coupling of
 15 4-[[4-(aminoiminomethyl)phenyl]amino]-4-oxobutanoic acid
 hydrochloride prepared in Example 1, Step 1 and 3-
 amino-4hydroxyfuran was dissolved in water (20 mL) and
 the pH adjusted to 10.5 by addition of LiOH · H₂O. The
 reaction was allowed to proceed at room temperature for
 20 2 hours and the product isolated by RPHPLC. The
 appropriate fractions were adjusted to pH 7 by addition
 of LiOH prior to solvent removal. Subsequent
 lyophilization gave a white solid:

¹H NMR (d₆ DMSO) δ 2.5 (m, 6H), 2.9 (m, 1H),
 25 4.08 (m, 1H), 4.42 (m, 2H), 7.80 (s, 4H), 8.5 (d, 1H),
 8.85 (s, 2H), 9.15 (s, 2H), 10.4 (s, 1H). MS (FAB)

337.1 (MH⁺), 319.2 (M - H₂O + H). Elemental analysis;

Required for C₁₅H₂₀N₄O₅ · CF₃CO₂H · 1.5 H₂O :

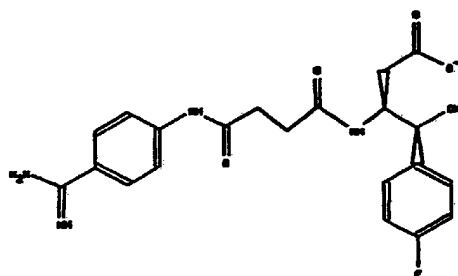
C 42.77 H 5.07 N 11.74

30 Found:

C 43.06 H 4.24 N 11.45

EXAMPLE 82a

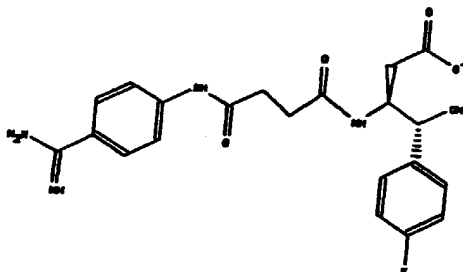
β (S)-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]- γ -hydroxy-(4-fluoro)phenylbutanoic acids (diastereoisomer A).



Hydrolysis of the cyclic ester prepared from 3-amino-4-hydroxyfuran and 4-[[4-(aminoiminomethyl)phenyl]amino]-4-oxobutanoic acid was carried out as described in Example 81 to give the desired hydroxy - acids with correct NMR, MS (FAB) 431.2 (MH⁺), and elemental analysis.

EXAMPLE 82b

β (S)-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]- γ -hydroxy-(4-fluorophenyl)butanoic acids (diastereoisomer B).



20

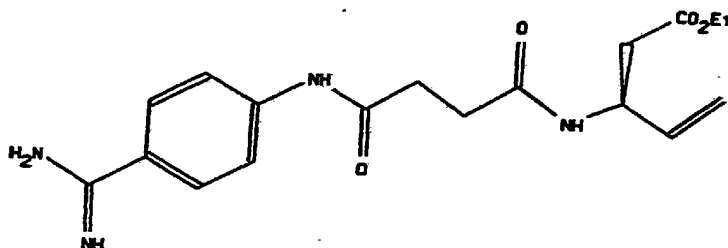
Hydrolysis of the cyclic ester prepared from 3-amino-4-hydroxyfuran and 4-[[4-(aminoiminomethyl)phenyl]amino]-4-oxobutanoic acid was carried out as described in Example 81 to give the desired hydroxy - acids with correct NMR, MS (FAB) 431.2 (MH⁺), and elemental analysis.

EXAMPLE 83

3-(S)-Ethyl 3-[[4-[[4-(aminoiminomethyl)phenyl]amino]-
1,4-dioxobutyl]amino]-4-pentenoate

5

10



15 The title compound was prepared in the manner of
Example 1 with the following modifications: Substituting
ethyl 3S-amino-4-pentenoate for 3-phenyl-beta-alanine.
The ethyl 3S-amino-4-pentenoate was prepared in an
analogous manner to the literature precedent as
20 described above and the structure was verified by C NMR
of the hydrochloride salt (CDCl₃) δ 14.9, 37.7, 51.4,
62.2, 121.9, 133.1, 171.0. Analysis of the beta amino
ester by chiral HPLC using a crownpak ether column
[CR(+)] cooled to 5°C using methanol:water 10:90 at pH
25 of 1 (HClO₄) and a flow rate of 0.5 mL/ min showed an
enantiomeric ratio of 100:0. The title compound had the
identical C NMR to the racemic material of Example 32.
The optical rotation of the TFA salt of the title
compound was: $[\alpha]_{589} +4.6$ (c 1.03, CH₃OH).

30

The following are prophetical examples.

EXAMPLE 84

5 ethyl [[[2[[[4-(aminoiminomethyl)
phenyl]amino]carbonyl]cyclopropyl]carbonyl]oxo]amino]-
4-pentynoate

Step 1 Preparation of ethyl [2-[[[4-(
(aminoiminomethyl)phenyl]amino]-1-oxo]cyclopropyl]-
10 carboxylate. Diethyl 1,2-cyclopropanedicarboxylate (25
g; trans isomer from Aldrich) is added to a solution of
5.65 g LiOH in 50 mL H₂O. The two phase mixture is
stirred and 50 mL ethanol added. After 5 min stirring,
a yellow homogeneous mixture is observed and stirring
15 continued for 24 h at 25°C. The crude reaction mixture
is partitioned between ethyl acetate and water (pH = 9).
The ethyl acetate extract should contain 15 g of a
mixture 2:1 of monoethyl ester: diacid. A portion of
this mixture (7.5 g) is suspended in dichloromethane and
20 treated with a total of 67 mL oxalyl chloride at room
temperature for a total time of 20 h. After
concentration in vacuo, the residual oil is taken up in
20 mL dimethylformamide and a mixture of
aminobenzamidine hydrochloride (12.5 g, 0.06 mol) and 15
25 mL of triethylamine in 50 mL dimethylformamide is slowly
added. After 16 hr stirring at 25°C, the reaction is
concentrated and the residue taken up in H₂O/acetonitrile
and purified by HPLC. The major peak (detection at 225
nM) is collected (Rt on a linear H₂O:ACN 5:95→70:30 over
30 25 min is 16 min). Lyophilization should give about 730
mg of a white powder which should give M+H at 276.2
(calculated for C₁₄H₁₇N₃O₃ : 275.1).

Step 2 Preparation of 2-[[[4-(
(aminoiminomethyl)phenyl]-amino]-1-
35 oxo]cyclopropyl]carboxylic acid. The product prepared
above is stirred in a solution of 1 g LiOH, 5mL
acetonitrile and 10 mL H₂O for 6 h at room temperature.

A precipitate should appear upon adjusting the pH to 6 and upon concentration. The precipitate is collected, redissolved in H₂O.acetonitrile and pH brought to 2 with HCl. This solution after lyophilization should give

5 about 480 mg of white solid : ¹H NMR (CD₃OD) δ 1.3 (m, 2H), 1.95 (m, 1H), 2.12 (m, 1H), 7.6 (m, 4H), .

Step 3 ethyl 3-[[2[[[[4(aminoiminomethyl)phenyl]amino]-1-oxo]cyclopropyl]-2-oxo]amino]-4-pentynoate.

10 The title compound is prepared in the manner of Example 1 substituting ethyl 3-amino-4-pentynoate for D-L-3-amino-3-phenylpropionic acid and 2-[[[4-(aminoiminomethyl)phenyl]amino]-oxo-]
15 [cyclopropylcarboxylic acid hydrochloride for 4-[[4-(aminoiminomethyl)phenyl]-amino-4-oxobutanoic acid hydrochloride. The product is verified by C NMR.

EXAMPLE 85

20 Ethyl 3-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-6-azido-4-hexynoate

The title compound can be prepared in the manner of Example 1 substituting ethyl 3-amino-6-azido-4-hexynoate for D,L-3-amino-3-phenylpropionic acid. The ethyl 3-
25 amino-6-azido-4-hexynoate can be prepared from the intermediate of Example 77, ethyl 3-amino-6-hydroxy-4-hexynoate, by standard methods: protection of the amine (BOC), mesylation of the alcohol, displacement by azide, and then deprotection of the amine (TFA). The product
30 can be purified by reverse phase HPLC using the conditions of Example 1 to afford the title compound. The product can be verified by C NMR.

EXAMPLE 86

35

3-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-6-azido-4-hexynoic acid

The title compound can be prepared by treating the final product of the previous example with porcine liver esterase in the manner of Example 29. The product can be purified by reverse phase HPLC using the conditions of Example 1 to afford the title compound. The product can be verified by C NMR.

EXAMPLE 87

Ethyl 3-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-6-amino-4-hexynoate

The title compound can be prepared by treating the title compound of Example 85 with triphenylphosphine and water in THF as described in the literature [N. Knouzi, M. Vaultier, R. Carrie Bull. of Chem. Soc. France, 815 (1985)] which can afford the title compound directly. The product can be purified by reverse phase HPLC using the conditions of Example 1 to afford the title compound. The product can be verified by C NMR.

20

EXAMPLE 88

3-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-6-amino-4-hexynoic acid

The title compound can be prepared by treating the final product of the previous example with porcine liver esterase in the manner of Example 29. The product can be purified by reverse phase HPLC using the conditions of Example 1 to afford the title compound. The product can be verified by C NMR.

30

EXAMPLE 89

Ethyl 3-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-6-methylsulfonamido-4-hexynoate

The title compound can be prepared in the manner of Example 1 substituting ethyl 3-amino-6-methylsulfonamido-4-hexynoate for D,L-3-amino-3-

phenylpropionic acid. The ethyl 3-amino-6-methylsulfonamido-4-hexynoate can be prepared from the intermediate of Example 85, ethyl N-BOC 3-amino-6-azido-4-hexynoate, by standard methods: reduction using triphenylphosphine and water in THF [N. Knouzi, M. Vaultier, R. Carrie Bull. of Chem. Soc. France, 815 (1985)], which can be followed by treatment with methanesulfonyl chloride and base, and deprotection (TFA). The product can be purified by reverse phase HPLC using the conditions of Example 1 to afford the title compound. The product can be verified by C NMR.

EXAMPLE 90

3-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-6-methylsulfonamido-4-hexynoic acid

The title compound can be prepared by treating the final product of the previous example with porcine liver esterase in the manner of Example 29. The product can be purified by reverse phase HPLC using the conditions of Example 1 to afford the title compound. The product can be verified by C NMR.

EXAMPLE 91

Ethyl 3-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-3-cyanopropanoate

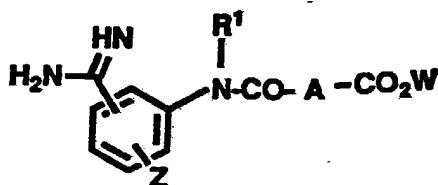
The title compound can be prepared in the manner of Example 1 substituting ethyl 3-amino-3-cyanopropanoate for D,L-3-amino-3-phenylpropionic acid. The ethyl 3-amino-3-cyanopropanoate can be prepared from ethyl N-CBZ-3-amino-3-carboxamidepropanoate using the following standard methods: dehydration to the nitrile (POCl₃), and deprotection (TFA). The product can be purified by reverse phase HPLC using the conditions of Example 1 to afford the title compound. The product can be verified by C NMR.

EXAMPLE 92

3-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-3-cyanopropanoate

- 5 The title compound can be prepared by treating the final product of the previous example with porcine liver esterase in the manner of Example 29. The product can be purified by reverse phase HPLC using the conditions of Example 1 to afford the title compound.
- 10 The product can be verified by C NMR.

This invention also relates to novel intermediate compounds formed when preparing the above described compounds which is represented by the chemical formula



wherein R^1 is selected from hydrogen, lower alkyl radicals, lower alkenyl radicals, aromatic hydrocarbon radicals, alicyclic hydrocarbon radicals, benzyl radicals, phenethyl radicals, where in all of said radicals are optionally substituted with halogen, lower alkoxy, hydroxy and lower alkyl;

A is selected from the group consisting of lower alkyl radicals, lower alkenyl radicals, lower alkynyl radicals, and alicyclic radicals;

Z is selected from the group consisting of hydrogen, lower alkyl, halogen, alkoxy, cyano, sulfonyl, carboxyl, and hydroxyl radicals.

W is as defined above but is preferably hydrogen or lower alkyl. Examples of these intermediates are described in Example 1, Step 1 and Example 2, Step 7 and Example 42, Step 1 and Example 51.

R^1 is preferably hydrogen. A is preferably lower alkyl. Z is preferably hydrogen.

In-Vitro Platelet Aggregation in PRP

Healthy male or female dogs were fasted for 8 hours prior to drawing blood; then 30 ml whole blood was collected using a butterfly needle and 30 cc plastic syringe with 3 ml of 0.129 M buffered sodium citrate (3.8%). The syringe was rotated carefully as blood was drawn to mix the citrate. Platelet-rich plasma (PRP) was prepared by centrifugation at 975 x g for 3.17 minutes at room temperature allowing the centrifuge to coast to a stop without braking. The PRP was removed from the blood with a plastic pipette and placed in a plastic capped, 50 mL Corning conical sterile centrifuge tube which was held at room temperature. Platelet poor plasma (PPP) was prepared by centrifuging the remaining blood at 2000 x g for 15 minutes at room temperature allowing the centrifuge to coast to a stop without braking. The PRP was adjusted with PPP to a count of $2-3 \times 10^8$ platelets per mL. 400 uL of the PRP preparation and 50 uL of the compounds solution to be tested or saline were preincubated for 1 minute at 37°C in a BioData, Horsham, PA). 50 uL of adenosine 5' diphosphate (ADP) (50 um final concentration) was added to the cuvettes and the aggregation was monitored for 1 minute. All compounds are tested in duplicate. Results are calculated as follows: Percent of control = $\frac{[(\text{maximal OD minus initial OD of compound}) \div (\text{maximal OD minus initial OD of control saline})] \times 100}{100}$. The % inhibition = $100 - (\text{percent of control})$.

The compounds tested and their median inhibitory concentrations (IC_{50}) are recorded in Table I. IC_{50} 's (dosage at which 50% of platelet aggregation is inhibited) were calculated by linear regression of the dose response curve. The assay results for the compounds of Examples 1 to 12 are set forth in Table I, below.

Table I

	Example	Dog PRP IC ₅₀ μ m	Ex Vivo Effect after IG Admins.
5	1	0.200	NT
	2	3.2	NT
	3	0.17	NT
	4	>10	+
10	5	>10	+
	6	0.7	NT
	7	NT	+
	8	>10	NT
15	9	>10	NT
	10	0.21	NT
	11	1.1	NT
	12	5.2	NT
20	13	>10	+
	14	0.4	NT
	15	1.3	NT
	16	0.39	NT
25	17	>10	+
	18	0.58	NT
	19	NT	-
	20	0.6	NT
30	21	>10	NT
	22	0.26	NT
	23	NT	NT
	24	>10	NT
35	25	>10	NT
	26	0.48	NT
	27	NT	NT
	28	NT	NT
40	29	1.5	NT
	30	NT	NT
	31	1.5	NT
	32	>10	+
45	33	0.27	NT
	34	>10	+
	35	0.36	NT
	36	NT	NT
50	37	0.29	NT
	38	3.0	+
	39	0.15	NT
	40	NT	NT
	41	0.19	NT
	42	NT	NT
	43	0.52	NT
	44	>10	NT
	45	4.8	NT
	46	NT	NT
	47	NT	NT
	48	1.7	NT
	49	.06	NT

	Example	Dog PRP IC ₅₀ μ M	Ex Vivo Effect after IG Admins.
5	50	0.053	NT
	51	1.8	NT
	52	NT	NT
	53	>10	NT
10	54	NT	NT
	55	>2.1	NT
	56	>10	NT
	57	NT	+
	58	0.11	NT
15	59	NT	NT
	60	>10	NT
	61	NT	NT
	62	NT	NT
	63	NT	NT
20	64	NT	NT
	65	NT	NT
	66	NT	NT
	67	NT	NT
	68	NT	NT
25	69	NT	NT
	70	0.13	+
	71	4.6	+
	72	0.07	+
	73	NT	NT
30	74	0.18	NT
	75	NT	NT
	76	0.6	NT
	77	NT	NT
	78	0.22	NT
35	79	NT	NT
	80	0.23	NT
	81	0.7	NT
	82a	0.15	NT
	82b	0.8	NT
40	83	NT	+

NT - non tested

INHIBITION OF EX VIVO COLLAGEN INDUCED AGGREGATION BY
COMPOUNDS OF THE INVENTION
PURPOSE

5 The purpose of this assay is to determine the effects of antiplatelet compounds on ex vivo collagen induced platelet aggregation when administered either intravenously or orally to dogs.

 Pretreatment (control) blood samples are
10 drawn from either conscious or anesthetized dogs (Beagles) and centrifuged to prepare platelet rich plasma (PRP). Aggregatory response to collagen is measured in a aggregometer and used as Control. Compounds are administered, either intragasterically
15 (either by capsule or stomach tube or intravenously). Blood samples are drawn at predetermined intervals after compound administration, PRP prepared and aggregation to collagen determined. Compound inhibition of aggregation is determined by comparing the aggregation response
20 after compound administration to the pretreatment response. The study is continued for a maximum of 24 hours or until the platelet aggregation returns to control levels. (If aggregation is still inhibited after 7 hours, a blood sample is drawn the following
25 morning and tested.) Duration of activity is determined by the length of time platelet aggregation is inhibited after compound administration.

 Compounds Example #32 and Example #38 were shown to inhibit platelet aggregation at 100% after
30 24 hours when administered orally to dogs at a dose of 20 mg/kg.

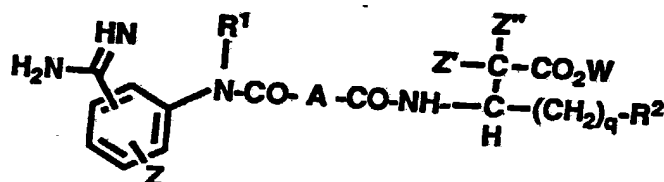
 Compound Example 13 was similarly shown to inhibit platelet aggregation at 100% when administered orally to dog at a dose of 12.5 mg/kg. In
35 addition when given orally at that dose for 10 (ten) consecutive days to dogs, compound Example 13 showed sustained antiaggregation activity.

Compounds of examples 71 and 72 are currently the best compounds.

From the foregoing description, one skilled in the art can easily ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions.

WHAT IS CLAIMED IS:

1. A substituted β amino acid derivative or a pharmaceutically acceptable salt, or prodrug thereof having the formula:



10

wherein R^1 is selected from the group consisting of hydrogen, lower alkyl radicals, lower alkenyl radicals, aromatic hydrocarbon radicals, alicyclic hydrocarbon radicals, benzyl radicals, phenethyl radicals, wherein said radicals are optionally substituted with halogen, lower alkoxy, hydroxy and lower alkyl;

R^2 is selected from the group consisting of hydrogen, lower alkyl radicals, lower alkenyl radicals, lower alkynyl radicals, alicyclic hydrocarbon radicals, aromatic hydrocarbon radicals, wherein said radicals are optionally substituted with hydroxy, lower alkoxy, lower alkyl, halogen, nitro, cyano, azido, ureido, ureylene, carboxyl or carbonyl derivatives, trifluoromethyl, acyloxy, alkylthio, arylthio, alkylsulfenyl, arylsulfenyl, alkylsulfonyl, arylsulfonyl, amino, alkylamino, trialkylsilyl, aminosulfonyl, dialkylamino, alkanoylamino, aroylamino, phenyl, naphthyl, lower alkynyl which are optionally substituted with one or more of the following: halogen, nitro, lower alkoxy, lower alkyl, trialkylsilyl, azide and phenyl.

A is selected from the group consisting of lower alkyl radicals, lower alkenyl radicals, lower alkynyl radicals, and alicyclic radicals, wherein said radicals are optionally substituted with hydroxyl, lower alkoxy, lower alkyl, halogen, alkoxycarbonylalkyl, amino,

alkylamino, dialkylamino, acylamino, alkylthio, sulfonyl, and aromatic hydrocarbons which are optionally substituted with halogen, nitro, lower alkoxy and lower alkyl;

- 5 W is selected from the group consisting of hydrogen, lower alkyl radicals, lower alkenyl radicals, lower alkynyl radicals, alicyclic hydrocarbon radicals and aromatic hydrocarbon radicals, wherein said radicals are optionally substituted with hydroxyl, lower alkoxy, 10 lower alkyl, halogen, nitro, amino, acyloxy, and phenyl and naphthyl which may be optionally substituted with halogen, nitro, lower alkoxy, and lower alkyl;

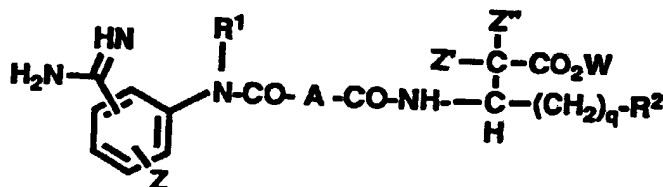
Z, Z', Z'' are independently selected from the group consisting of hydrogen, lower alkyl radicals, halogen, 15 alkoxy, cyano, sulfonyl, carboxyl, alkoxycarbonyl, and hydroxyl radicals;

q is an integer from 0 to about 6; and

with the proviso that when A is trimethylene and q is 0 then R₂ is not hydrogen, methyl radical or phenyl 20 radical and also that when A is trimethylene and q is 1 then R₂ is not hydrogen.

2. A substituted β amino acid derivative or a pharmaceutically acceptable salt thereof having the formula:

25



30

wherein R¹ is selected from the group consisting of hydrogen, lower alkyl radicals, lower alkenyl radicals, aromatic hydrocarbon radicals, alicyclic hydrocarbon 35 radicals, benzyl radicals, phenethyl radicals, wherein said radicals are optionally substituted with halogen, alkoxy, hydroxy and lower alkyl;

R^2 is selected from the group consisting of hydrogen, lower alkyl radicals, lower alkenyl radicals, lower alkynyl radicals, alicyclic hydrocarbon radicals, aromatic hydrocarbon radicals, wherein said radicals are optionally substituted with hydroxy, lower alkoxy, lower alkyl, halogen, nitro, cyano, azido, ureide, ureylene, carboxyl or carbonyl derivatives, trifluoromethyl, acyloxy, alkylthio, arylthio, alkylsulfenyl, arylsulfenyl, alkylsulfonyl, arylsulfonyl, amino, alkylamino, trialkylsilyl, sulfonylamino, dialkylamino, alkanoyl amino, aroylamino, phenyl, naphthyl and lower alkynyl which are optionally substituted with one or more of the following: halogen, nitro, lower alkoxy, lower alkyl, trialkyl silyl, azide and phenyl.

A is selected from the group consisting of lower alkyl radicals, lower alkenyl radicals, lower alkynyl radicals, and alicyclic radicals, wherein said radicals are optionally substituted with hydroxyl, lower alkoxy, lower alkyl, halogen, alkoxycarbonylalkyl, amino, alkylamino, dialkylamino, acylamino, alkylthio, sulfonyl, and aromatic hydrocarbons which are optionally substituted with halogen, nitro, lower alkoxy and lower alkyl;

W is selected from the group consisting of hydrogen, lower alkyl radicals, lower alkenyl radicals, lower alkynyl radicals, alicyclic hydrocarbon radicals and aromatic hydrocarbon radicals, wherein said radicals are optionally substituted with hydroxyl, lower alkoxy, lower alkyl, halogen, nitro, amino, acyloxy, and phenyl and naphthyl which may be optionally substituted with halogen, nitro, lower alkoxy, and lower alkyl;

Z, Z', Z" are independently selected from the group consisting of hydrogen, lower alkyl radicals, halogen, alkoxy, cyano, sulfonyl, carboxyl, alkoxycarbonyl, and hydroxyl radicals;

q is an integer from 0 to about 6; and

with the proviso that when A is trimethylene and q is 0 then R₂ is not hydrogen, methyl radical or phenyl radical and also that when A is trimethylene and q is 1 then R₂ is not hydrogen.

- 5 3. The substituted β amino acid derivatives as recited in Claim 1 wherein;

R₁ is selected from hydrogen, lower alkyl radicals of 1 to about 6 carbon atoms, lower alkenyl radicals of 1 to about 6 carbon atoms, aromatic hydrocarbon
10 radicals, alicyclic hydrocarbon radicals of 3 to about 6 carbon atoms, benzyl radicals, phenethyl radicals, wherein said radicals are optionally substituted with halogen, lower alkoxy, hydroxy and lower alkyl;

R₂ is selected from hydrogen, lower alkyl radicals
15 of 1 to about 6 carbon atoms, lower alkenyl radicals of 2 to about 6 carbon atoms, lower alkynyl radicals of 2 to about 8 carbon atoms, alicyclic hydrocarbon radicals of 3 to 6 carbon atoms, aromatic hydrocarbon radicals, wherein said radicals are optionally substituted with
20 hydroxyl, lower alkoxy, lower alkyl, halogen, nitro, cyano, azido, ureide, ureylene, amino, trialkylsilyl, alkylsulfonyl, phenylsulfonyl, trifluoromethyl, acetoxy, acetylamino, benzoylamino, carbonyl, carboxyl
25 amino;

A is selected from lower alkyl radicals of 1 to about 6 carbon atoms, lower alkenyl radicals of 2 to about 6 carbon atoms, lower alkynyl radicals of 2 to about 4 carbon atoms, and alicyclic hydrocarbon radicals
30 of 3 to about 5 carbon atoms, wherein said radicals are optionally substituted with hydroxyl, lower alkoxy, halogen, alkylthio and amino;

W is selected from hydrogen, lower alkyl radicals of 1 to about 6 carbon atoms, lower alkenyl radicals of 2
35 to about 6 carbon atoms, alicyclic hydrocarbon radicals of 3 to about 6 carbon atoms, and aromatic hydrocarbon radicals of 6 to about 12 carbon atoms, wherein all of

said radicals are optionally substituted with hydroxyl, lower alkoxy, lower alkyl, halogen, nitro, amino, and acyloxy;

5 Z, Z', Z" are independently selected from the group consisting of hydrogen, halogen, alkoxy, cyano, sulfonyl, carboxyl, alkoxycarbonylalkyl, alkoxycarbonyl and lower alkyl radicals; and

q is an integer from 0 to about 6.

4. A substituted β amino acid derivative as recited in
10 claim 1 wherein;

R₁ is selected from hydrogen, lower alkyl radicals, lower alkenyl radicals, aromatic hydrocarbon radicals, alicyclic hydrocarbon radicals, benzyl radicals, phenethyl radicals, wherein all of said radicals are
15 optionally substituted with halogen, lower alkoxy, hydroxy and lower alkyl;

R² is selected from hydrogen, lower alkyl radicals, lower cycloalkyl radicals, lower alkenyl radicals, lower alkynyl radicals, phenol radicals, phenyl radicals,
20 naphthyl radicals wherein each radical may have one or more substituents selected from the group consisting of halogen, lower alkyl, lower alkoxy, carboxyl derivatives, nitro, cyano, azido, ureide, ureylene, alkylcarbonyloxy, hydroxyl, alkylamino, alkoxycarbonyl,
25 trialkylsilyl, alkoxyimino, alkylsulfonyl, phenylsulfonyl, alkylsulfonyl amino, phenylsulfonyl amino and amino;

A is selected from lower alkyl radicals, lower cycloalkyl, and lower alkenyl radicals;

30 W is selected from the group consisting of hydrogen and lower alkyl radicals;

Z, Z', Z" are independently selected from the group consisting of halogen, hydrogen, and lower alkoxy, and alkoxycarbonyl, and alkoxycarbonylmethyl, and lower
35 alkyl radicals;

q is an integer from 0 to about 6.

5. A substituted β amino acid derivative as recited in Claim 1 wherein;

R^1 is selected from hydrogen, lower alkyl radicals, benzyl radicals, phenyl radicals;

- 5 R^2 is selected from hydrogen, lower alkyl radicals, lower alkenyl, lower alkynyl, lower cycloalkyl, phenyl and benzyl radicals wherein said radicals are optionally substituted with carboxyl, alkoxycarbonyl, cyano, hydroxyaminocarbonyl, hydroxyl, ureide,
- 10 alkoxymethylaminocarbonyl, halogen, acetoxy, alkoxy, methoxyimino, azido, trimethylsilyl, phenylsulfonyl, methylsulfonyl amino, phenylsulfonyl amino, phenyl and t-butyl;

- A is selected from lower alkyl radicals, lower
- 15 cycloalkyl and lower alkenyl radicals wherein said radicals can be optionally substituted with lower alkyl radicals;

W is selected from the group consisting of hydrogen and lower alkyl radicals;

- 20 Z, Z', Z'' are independently selected from the group of hydrogen, methyl, ethyl, hydroxy, methoxy, chloro, fluoro, alkoxycarbonyl, and alkoxycarbonylmethyl;

q is an integer from 0 to about 4.

6. A substituted β amino acid as recited in Claim 1
- 25 wherein;

R^1 is selected from hydrogen and benzyl radicals;

- R^2 is selected from hydrogen and lower alkyl radicals wherein said lower alkyl radicals can be optionally substituted with carboxyl, alkoxycarbonyl,
- 30 cyano, hydroxyaminocarbonyl, hydroxyl, ureide alkoxymethylaminocarbonyl, acetyloxy, alkoxy, methoxyimino, azido, trimethylsilylethynyl, and phenylsulfonyl;

- A is selected from lower alkyl radicals and is
- 35 optionally substituted with lower alkyl radicals;

W is selected from the group consisting of hydrogen and lower alkyl;

Z is selected from hydrogen, halogen and lower alkyl;

Z' and Z'' are independently selected from hydrogen lower alkyl and hydroxy;

5 Q is 0 to about 2.

7. A substituted β amino acid as recited in Claim 1 wherein;

R¹ is selected from hydrogen and benzyl radicals;

10 R² is selected from hydrogen and lower alkyl radicals wherein said lower alkyl radicals can be optionally substituted with carboxyl, alkoxycarbonyl, cyano, hydroxyaminocarbonyl, hydroxyl, ureide, alkoxymethylaminocarbonyl, acetoxy, alkoxy, methoxyimino, azido, trimethylsilylethynyl, and
15 phenylsulfonyl;

A is lower cycloalkyl radicals;

W is selected from the group consisting of hydrogen and lower alkyl;

20 Z is selected from hydrogen, halogen and lower alkyl;

Z' and Z'' are independently selected from hydrogen lower alkyl and hydroxy;

Q is 0 to about 2.

25 8. A substituted β amino acid as recited in Claim 1 wherein;

R¹ is selected from hydrogen and benzyl radicals;

R² is selected from lower alkenyl and lower alkynyl;

A is selected from lower alkyl and may be substituted with lower alkyl;

30 W is selected from the group consisting of hydrogen and lower alkyl radicals;

Z is selected from hydrogen, halogen and lower alkyl;

35 Z' and Z'' are independently selected from hydrogen, lower alkyl and hydroxy;

Q is an integer from 0 to about 2.

9. A substituted β amino acid as recited in Claim 1 wherein;
R¹ is selected from hydrogen and benzyl radicals;
R² is selected from lower alkenyl and lower alkynyl;
5 A is selected from cycloalkyl;
W is selected from the group consisting of hydrogen and lower alkyl radicals;
Z is selected from hydrogen, halogen and lower alkyl;
10 Z' and Z'' are independently selected from hydrogen, lower alkyl and hydroxy;
Q is an integer from 0 to about 2.
10. A substituted β amino acid derivative as recited in Claim 5 wherein "A" is selected from the group of
15 methylenyl, propylenyl, vinylenyl, allylenyl, ethylidenyl, and ethylenyl radicals.
11. A substituted β amino acid derivative as recited in Claim 5 which is Dimethyl3[[4-[(4-(aminoiminomethyl)phenyl)-amino]-1,4-dioxobutyl] amino]pentanedioate.
- 20 12. A substituted β amino acid as recited in Claim 5 which is
3-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]pentanedioic acid, monomethylester.
13. A substituted β amino acid as recited in Claim 5
25 which is
3-[[4-[[4-(aminoiminomethyl)phenyl]-amino]-1,4-dioxobutyl]amino]pentanedioic acid.
14. A substituted β amino acid as recited in Claim 5 which is
30 Ethyl3-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-4-phenylsulfonyl butanoate.
15. A substituted β amino acid as recited in Claim 5 which is
3-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl
35]amino]-4-phenylsulfonyl butanoic acid.
16. A substituted β amino acid derivative as recited in Claim 5 which is Ethyl 3-[[4-[[4-

- (aminoiminomethyl)phenyl]amino)-1,4-dioxobutyl]amino]-5-hexenoate.
17. A substituted β amino acid derivative as recited in Claim 5 which is 3-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1, 4-dioxobutyl]amino]4-pentenoic acid.
18. A substituted β amino acid derivative as recited in Claim 5 which is Ethyl 3-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1, 4-dioxobutyl]amino]-4-pentynoate.
19. A substituted β amino acid derivative as recited in Claim 5 which is 3-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1, 4-dioxobutyl]amino]-4-pentynoic acid.
20. A substituted β amino acid derivative as recited in Claim 5 which is (3S)-Ethyl 3-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-4-pentynoate.
21. A substituted β amino acid derivative as recited in Claim 5 which is (3S) -3,[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-4-pentynoic acid.
22. A substituted β amino acid derivative as recited in Claim 5 which is
- 25 t-Butyl β -[[[2-[[[4-(aminoiminomethyl)phenyl]amino]carbonyl] cyclopropyl]carbonyl]amino]phenylpropanoate
23. A substituted β amino acid derivative as recited in Claim 5 which is ethyl
- 30 β -[[[2-[[[4-(aminoiminomethyl)phenyl]amino]carbonyl]cyclopropyl]carbonyl]amino]butanoate, isomer 1.
24. A substituted β amino acid derivative as recited in Claim 5 which is ethyl
- 35 β -[[[2-[[[4-(aminoiminomethyl)phenyl]amino]carbonyl]cyclopropyl]carbonyl]amino]butanoate, isomer 2.

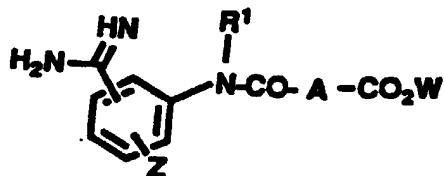
25. A pharmaceutical composition comprising at least one non-toxic pharmaceutically acceptable carrier and at least one compound according to Claim 1.

26. A pharmaceutical composition as recited in claim 25 comprising at least one non-toxic pharmaceutically acceptable carrier and at least one compound according to Claim 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 or 24 together with said carrier.

27. A method of treating a mammal to inhibit platelet aggregation comprising administering a therapeutically effective amount of at least one compound of Claim 1 to a mammal in need of such treatment.

28. A method of treating a mammal to inhibit platelet aggregation as recited in claim 27 comprising administering a therapeutically effective amount of the compound of Claim 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 or 24 to a mammal in need of such treatment.

28. A compound with the formula:



25

wherein R¹ is selected from the group consisting of hydrogen, lower alkyl radicals, lower alkenyl radicals, alicyclic hydrocarbon radicals, aromatic hydrocarbon radicals, benzyl radical and phenethyl radicals;

A is selected from the group consisting of lower alkyl radicals, lower alkenyl radicals, lower alkynyl radicals, and alicyclic radicals;

Z, is selected from the group consisting of hydrogen, lower alkyl, halogen, alkoxy, cyano, sulfonyl, carboxyl, and hydroxyl radicals;

W is hydrogen and lower alkyl.

INTERNATIONAL SEARCH REPORT

PCT/US 92/08512

International Application No

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) ⁶		
According to International Patent Classification (IPC) or to both National Classification and IPC Int.Cl. 5 A61K31/21; C07C257/18		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
Int.Cl. 5	A61K ; C07C	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸		
III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹		
Category *	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
A	EP,A,0 445 796 (F. HOFFMANN-LA ROCHE AG) 11 September 1991 cited in the application see the whole document ---	1-26,29
A	EP,A,0 381 033 (F. HOFFMANN-LA ROCHE AG) 8 August 1990 cited in the application see claims 1,9,11-13 ---	1-26,29
A	EP,A,0 372 486 (F. HOFFMANN-LA ROCHE AG) 13 June 1990 cited in the application see the whole document ---	1-26,29
		-/--
<p>⁹ Special categories of cited documents: ¹⁰</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"A" document member of the same patent family</p>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search 08 JANUARY 1993		Date of Mailing of this International Search Report 28. 01. 93
International Searching Authority EUROPEAN PATENT OFFICE		Signature of Authorized Officer P. BOSMA

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	EP,A,0 352 249 (MONSANTO COMPANY) 24 January 1990 see the whole document & US,A,4 879 313 cited in the application	1-26,29

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 92/08512

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 27 and 28 are directed to a method of treatment of (diagnostic method practised on) the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO. US 9208512
SA 65562

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
 The members are as contained in the European Patent Office EDP file on
 The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 08/01/93

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0445796	11-09-91	JP-A-	4217652	07-08-92
EP-A-0381033	08-08-90	AU-A-	4881790	09-08-90
		CA-A-	2008311	31-07-90
		JP-A-	2235853	18-09-90
		US-A-	5084466	28-01-92
EP-A-0372486	13-06-90	AU-A-	4586589	01-11-90
		CA-A-	2004127	08-06-90
		JP-A-	2223543	05-09-90
		US-A-	5039805	13-08-91
EP-A-0352249	24-01-90	US-A-	4879313	07-11-89
		JP-A-	2078653	19-03-90
		US-A-	4992463	12-02-91
		US-A-	5037808	06-08-91
		US-A-	5053393	01-10-91
		US-A-	5091396	25-02-92

EPO FORM P007

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82